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(54) **ISOLATION OF A DUAL COX-2 AND 5-LIPDXYGENASE INHIBITOR FROM *ACACIA***

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(56) **References Cited**

U.S. PATENT DOCUMENTS

3,686,872	A	8/1972	Whitworth et al.
3,706,581	A	12/1972	Whitworth et al.
4,035,510	A	7/1977	Van Scott et al.
4,268,517	A	5/1981	Niebes et al.
4,374,824	A	2/1983	Wahmi
4,515,804	A	5/1985	Marti et al.
4,627,977	A	12/1986	Gaffar et al.
4,946,684	A	8/1990	Blank et al.
4,965,067	A	10/1990	Wietfeldt
5,096,701	A	3/1992	White, Jr. et al.
5,098,709	A	3/1992	Kang
5,156,835	A	10/1992	Nabi et al.
5,437,856	A	8/1995	Lukacovic et al.
5,443,983	A	8/1995	Ochoa et al.
5,470,589	A	11/1995	Shi
5,545,411	A	8/1996	Chancellor
5,585,371	A	12/1996	Lardy
5,589,160	A	12/1996	Rice
5,605,929	A	2/1997	Liao et al.
5,643,598	A	7/1997	Meybeck
5,650,432	A	7/1997	Walker et al.
5,650,433	A	7/1997	Watanabe et al.
5,651,987	A	7/1997	Fuisz
5,756,538	A	5/1998	Cassels et al.
5,766,614	A	6/1998	Yong
5,773,014	A	6/1998	Perrier et al.
5,795,911	A	8/1998	Cheng et al.
5,804,168	A	9/1998	Murad
5,852,057	A	12/1998	Muto et al.
5,858,371	A	1/1999	Singh et al.
5,886,029	A	3/1999	Dhaliwal
5,886,155	A	3/1999	Armah et al.
5,908,628	A	6/1999	Hou
5,922,756	A	7/1999	Chan
5,962,517	A	10/1999	Murad
5,968,973	A	10/1999	Cheng et al.
6,080,401	A	6/2000	Reddy et al.
6,083,921	A	7/2000	Xu
6,093,403	A	7/2000	Huo et al.
6,113,909	A	9/2000	Han et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CA	2 126 513	A1	1/1995
CA	2 451 844	A1	1/2003

(Continued)

OTHER PUBLICATIONS

Colby, "Calculating Synergistic and Antagonistic Responses of Herbicide Combinations," *Weeds 15*(1): 20-22, 1967.

Kowal-Bielecka et al., "Evidence of 5-lipoxygenase overexpression in the skin of patients with systemic sclerosis: a newly identified pathway to skin inflammation in systemic sclerosis," *Arthritis Rheum, 44*(8): 1865-1875, 2001.

Martel-Pelletier et al., "Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs," *Ann. Rheum. Dis. 62*:501-509, 2003.

RELEVE Joint Support Formula Product Information, retrieved Nov. 6, 2002, from http://www.fitnessfirstusa.com/details.asp?item=8244, 3 pages.

(Continued)

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(57) **ABSTRACT**

The present disclosure provides compositions and methods for alleviating inflammatory-associated diseases and conditions. These methods include administering individual and/or a mixture of multiple flavans isolated from a single or multiple *Acacia* genus of plants. The present disclosure also includes methods for isolating and purifying from an *Acacia* genus of plants a composition of flavans having dual specificity for cyclooxygenase (COX-2) and 5-lipoxygenase (5-LO) enzymes.

8 Claims, 14 Drawing Sheets

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,126,940	A	10/2000	Takahashi et al.
6,126,950	A	10/2000	Bindra et al.
6,193,977	B1	2/2001	Han et al.
6,194,469	B1	2/2001	Nair et al.
6,197,808	B1	3/2001	Cheng et al.
6,217,875	B1	4/2001	Murai et al.
6,221,341	B1	4/2001	Montgomery
6,235,294	B1	5/2001	Perrier et al.
6,241,972	B1	6/2001	Herms et al.
6,248,341	B1	6/2001	Anderson et al.
6,264,926	B1	7/2001	Farooqi et al.
6,264,995	B1	7/2001	Newmark et al.
6,280,751	B1	8/2001	Fletcher et al.
6,290,995	B1	9/2001	Xinxian
6,319,523	B1	11/2001	Zhou
6,333,304	B1	12/2001	Bath et al.
6,387,416	B1	5/2002	Newmark et al.
6,391,346	B1	5/2002	Newmark et al.
6,391,872	B1	5/2002	Marfat
6,475,530	B1	11/2002	Kuhrts
6,555,573	B2	4/2003	Rosenbloom
6,576,660	B1	6/2003	Liao et al.
6,685,971	B2	2/2004	Xu
7,045,158	B2	5/2006	Wolfson et al.
7,074,438	B2	7/2006	Xu
7,108,868	B2	9/2006	Jia et al.
7,189,385	B2	3/2007	Montgomery
7,192,611	B2	3/2007	Jia et al.
7,514,469	B2	4/2009	Jia
7,531,521	B2	5/2009	Burnett et al.
7,615,239	B2	11/2009	Santo et al.
7,674,830	B2	3/2010	Jia
7,695,743	B2	4/2010	Jia et al.
7,897,182	B2	3/2011	Woo et al.
7,972,632	B2	7/2011	Jia
8,034,387	B2	10/2011	Jia et al.
8,124,134	B2	2/2012	Jia et al.
8,148,416	B2	4/2012	El-Naggar et al.
8,568,799	B2	10/2013	Jia et al.
8,652,535	B2	2/2014	Jia et al.
2001/0002407	A1	5/2001	Nair et al.
2001/0026813	A1	10/2001	Kim et al.
2001/0046963	A1	11/2001	Wenzel et al.
2002/0086070	A1	7/2002	Kuhrts
2002/0122836	A1	9/2002	Obukowicz et al.
2002/0136784	A1	9/2002	Obukowicz et al.
2002/0146467	A1	10/2002	Jung et al.
2003/0045562	A1	3/2003	El-Naggar et al.
2003/0105030	A1	6/2003	Liao et al.
2003/0113797	A1	6/2003	Jia et al.
2003/0125264	A1	7/2003	Malik
2003/0165588	A1	9/2003	Jia et al.
2003/0166583	A1	9/2003	Yoa-Pu Hu et al.
2003/0170186	A1	9/2003	Geers et al.
2003/0203857	A1	10/2003	Ohnogi et al.
2004/0057908	A1	3/2004	Bowen et al.
2004/0185124	A1	9/2004	Hayashi
2004/0220119	A1	11/2004	Jia
2005/0049206	A1	3/2005	Gong et al.
2005/0096281	A1	5/2005	Jia et al.
2006/0008749	A1	1/2006	Sobel et al.
2006/0079467	A1	4/2006	Jia et al.
2006/0140881	A1	6/2006	Xu et al.
2006/0141073	A1	6/2006	Worrell et al.
2006/0177528	A1	8/2006	Jia
2006/0204596	A1	9/2006	Jia et al.
2007/0065524	A1	3/2007	Wang
2007/0264361	A1	11/2007	Jo et al.
2008/0107759	A1	5/2008	Woo et al.
2008/0176811	A1	7/2008	Geers et al.
2008/0214658	A1	9/2008	Woo et al.
2008/0279969	A1	11/2008	Jo et al.
2009/0304830	A1	12/2009	Jo et al.
2011/0117224	A1	5/2011	Woo et al.
2011/0207806	A1	8/2011	Jia
2011/0245333	A1	10/2011	Jia et al.
2012/0053138	A1	3/2012	Jia et al.

FOREIGN PATENT DOCUMENTS

CA	2 484 192	A1	11/2003
CN	1057196	A	12/1991
CN	1093914	A	10/1994
CN	1096680	A	12/1994
CN	1101856	A	4/1995
CN	1189365	A	8/1998
CN	1043406	C	5/1999
CN	1228968	A	9/1999
CN	1285202	A	2/2001
EP	0 296 625	A2	12/1988
EP	0 633 022	A2	1/1995
EP	0 742 012	A2	11/1996
EP	0 633 022	B1	2/1997
EP	0 956 867	A1	11/1999
EP	1 147 764	A2	10/2001
FR	2 651 132	A1	3/1991
FR	2 687 572	A1	8/1993
GB	2 024 817	A	1/1980
GB	2 306 321	A	5/1997
JP	57-038721	A	3/1982
JP	61-050921	A	3/1986
JP	61-083179	A	4/1986
JP	61-161219	A	7/1986
JP	61-238719	A	10/1986
JP	63-027435	A	2/1988
JP	03-240725	A	10/1991
JP	03-251518	A	11/1991
JP	05-271088	A	10/1993
JP	05-331061	A	12/1993
JP	07-010768	A	1/1995
JP	07-017847	A	1/1995
JP	07-025761	A	1/1995
JP	07-055895	B2	6/1995
JP	07-165598	A	6/1995
JP	07-223941	A	8/1995
JP	07-242555	A	9/1995
JP	07-277942	A	10/1995
JP	08-026969	A	1/1996
JP	08-104628	A	4/1996
JP	09-227374	A	9/1997
JP	10-025238	A	1/1998
JP	10-130162	A	5/1998
JP	10-182415	A	7/1998
JP	10-287528	A	10/1998
JP	11-140497	A	5/1999
JP	2000-044481	A	2/2000
JP	2000-506901	A	6/2000
JP	2000-226329	A	8/2000
JP	2000-31954	A	11/2000
JP	2001-220353	A	8/2001
JP	2002-053484	A	2/2002
JP	2002-080362	A	3/2002
JP	2003-002820	A	1/2003
JP	2003-081746	A	3/2003
JP	2003-212771	A	7/2003
JP	2003-212786	A	7/2003
JP	2004-244385	A	9/2004
KR	1996-0003725	A	2/1996
KR	1996-0040370	A	12/1996
KR	2001-0017481	A	3/2001
KR	2001-0069130	A	7/2001
KR	2002-0013675	A	2/2002
KR	2002-0031608	A	5/2002
KR	2003-0021640	A	3/2003
KR	10-0465113	B1	12/2004
KR	10-0522579	B1	10/2005
WO	97/36497	A2	10/1997
WO	98/19651	A1	5/1998
WO	98/40086	A2	9/1998
WO	98/42363	A1	10/1998
WO	98/49256	A1	11/1998
WO	00/59523	A1	10/2000
WO	00/67749	A1	11/2000

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

WO	00/74662	A2	12/2000
WO	01/30341	A1	5/2001
WO	02/07745	A1	1/2002
WO	02/09699	A2	2/2002
WO	02/39973	A2	5/2002
WO	02/42429	A2	5/2002
WO	02/47615	A2	6/2002
WO	03/002134	A1	1/2003
WO	03/009825	A2	2/2003
WO	03/015766	A1	2/2003
WO	03/024470	A1	3/2003
WO	03/082312	A1	10/2003
WO	03/092599	A2	11/2003
WO	2004/075844	A2	9/2004
WO	2004/089392	A1	10/2004
WO	2005/020932	A2	3/2005
WO	2006/099217	A2	9/2006

OTHER PUBLICATIONS

Sánchez-Borges et al., "NSAID Hypersensitivity in the COX-2 Inhibitor Era," *ACI International 13*:211-218, 2001.

Whelton et al., "Cyclooxygenase-2 Specific Inhibitors and Cardiorenal Function: A Randomized Controlled Trial of Celecoxib and Rofecoxib in Older Hypertensive Osteoarthritis Patients," *Am. J. Ther. 85*:85-95, 2001.

Whitaker "The Hidden 'Pain Trigger'," Advances in Pain Relief Research, Spring, 2003, 24 pages.

"L17—(baicalin near20 catcchin) near20 weight" Search History, retrieved Jan. 8, 2009, from http://jupiter1:42900/bin/cgi-bin/PreSearch.p1, 1 page.

"Move Free Advanced Ingredients—Uniflex," retrieved May 7, 2008, from http://www.movefreeadvanced.com/ingredients.asp?q=uniflex, 3 pages.

"*Scutellaria* Root/ *Official Monographs for Part II,*" in The Japanese Pharmacopoeia, 14th ed. (English version), Society of Japanese Pharmacopoeia, Tokyo, Japan, 2001, pp. 1042-1043.

Abdulrazak et al., "Chemical Composition, Phenolic Concentration and In Vitro Gas Production Characteristics of Selected *Acacia* Fruits and Leaves," *Asian-Aus J Anim Sci 13*(7):935-940, 2000.

Afolayan et al., "The antimicrobial activity 3,5,7-trihydroxyflavone isolated from the shoots of *Helichrysum aureonitens," Journal of Ethnopharmacol. 57*(3):177-181, 1997.

Agarwal et al., "Protection Against Ultraviolet B Radiation-Induced Effects in the Skin of SKH-1 Hairless Mice by a Polyphenolic Fraction Isolated From Green Tea," *Photochemistry and Photobiology 58*(5): 695-700, Nov. 1993.

Amos et al., "The Pharmacological Effects of an Aqueous Extract from *Acacia nilotica* Seeds," *Phytotherapy Research 13*:683-685,1999.

Ardlie et al., "Effects of Trifluoperazine of Platelet Activation," *Thromb. Res. 38*(6):695-706, 1985.

Arnaud, "COX-2: an in vivo evidence of its participation in heat stress-induced myocardial preconditioning," *Cardiovas Res 58*:582-588, 2003.

Asaki et al., "The Effect of Oral Rinses Extracted from Japanese Tea on the Experimental Gingivitis in Man," *Journal of the Japanese Association of Peridontology 37*(2):412-421, 1995, (Abstract only).

Azad et al., "Isolation of (+)—catechin and a new polyphenolic compound in Bengal catechu," *J Wood Sci 47*(5): 406-409, 2001.

Babu et al., "Aspirin and Asthma," *Chest 118*:1470-1476, 2000.

Bastianetto et al., "Neuroprotective abilities of resveratrol and other red wine constituents against nitric oxide-related toxicity in cultured hippocampal neurons," *British Journal of Pharmacology 131*:711-720, 2000.

Baumann et al., "Flavonoids and Related Compounds as Inhibitors of Arachidonic Acid Peroxidation," *Prostaglandins 20*(4): 627-639, Oct. 1980.

Bertolini et al., "Dual Acting Anti-Inflammatory Drugs: A Reappraisal," *Pharmacol Res 44*(6):437-450, 2001.

Bhagwat et al., "Flavanoid composition of tea: Comparison of black and green teas," 2003 IFT Annual Meeting and Food Expo, Jul. 12-16, 2003, Chicago, IL, 1 page (Poster).

Bhāvamiśra; Bhāvaprakāśa—Edited & translated by Brahmashankara Misra & RupaLalaji Vaisya, Part-I: Chaukhambha Sanskrit Sansthan, Varanasi, Edn. 9th, Time of origin 16th century, p. 110, Formulation ID: RS/3007D, Formulation Name: Dantakūrcikā(04), 3 pages. (with English translation).

Bickford et al., "Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats," *Brain Res 866*:211-217, Jun. 2, 2000.

Bickford et al., "Effect of Normobaric Hyperoxia on Two Indexes of Synaptic Function in Fisher 344 Rats," *Free Rad Biol Med 26*(7/8):817-824, Apr. 1999.

Bickford et al., "Effects of aging on cerebellar nonradrenergic function and motor learning: nutritional interventions," *Mech Ageing Dev 111*:141-154, Nov. 1999.

Boozer et al., "An herbal supplement containing Ma Huang-Guarana for weight loss: a randomized, double-blind trial," *International Journal of Obesity 25*: 316-324, 2001.

Bossett et al., "Photoageing shows histological features of chronic skin inflammation without clinical and molecular abnormalities," *Brit J Dermatol 149*:826-835, 2003.

Boumendjel et al., "B-ring substituted 5,7 Dihydroxyflavonols with High-Affinity Binding to a P-Glycoprotein Responsible for Cell Multidrug Resistance," *Bioorg. Med. Chem. Lett. 11*(1):75-77, 2001.

Bridaeu et al., "A Human Whole Blood Assay for Clinical Evaluation of Biochemical Efficacy of Cyclooxygenase Inhibitors," *Inflamm. Res. 45*:68-74, 1996.

Brock et al., "Arachidonic Acid is Preferentially Metabolized by Cyclooxygenase-2 to Prostacyclin and Prostaglandin E_2," *J Biol Chem 274*(17):11660-11666, Apr. 1999.

Bunting et al., "The Prostacyclin-Thromboxane A_2 Balance: Pathophysiological and Therapeutic Implications," *Brit Med Bull 39*(3):271-276, 1983.

Butenko et al., "Anti-inflammatory properties and inhibiton of leukotriene C_4 Biosynthesis in vitro by flavonoid baicalein from *Scutellaria baicalensis* georgy roots," *Agents Actions 39, Special Conference Issue*: C49-C50, 1993.

Butterfield et al., "Structural and Functional Changes in Proteins Induced by Free Radical-mediated Oxidative Stress and Protective Action of the Antioxidants *N-tert*-Butyl-α-phenylnitrone and Vitamine E^{a}," *Ann NY Acad Sci 854*:448-462, Nov. 20, 1998.

Cao et al., "Hyperoxia-induced changes in antioxidant capacity and the effect of dietary antioxidants," *J Applied Physiol 86*(6):1817-1822, Jun. 1999.

Cao et al., "Oxygen-Radical Absorbance Capacity Assay for Antioxidants," *Free Radical Biology &Medicine 14*:303-311, 1993.

Carney et al., "Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound *N-tert*-butyl-α-phenylnitrone," *Proc. Natl. Acad. Sci. USA 88*:3633-3636, May 1991.

Carson et al., "The cellular response in neruoinflammation: The role of leukocytes, microglia and astrocytes in neuronal death and survival," *Clin Neurosci Res 6*(5):237-245, Dec. 2006.

Cartford et al., "Eighteen-Month-Old Fischer 344 Rats Fed a Spinach-Enriched Diet Show Improved Delay Classical Eyeblink Conditioning and Reduced Expression of Tumor Necrosis Factor α(TNFα) and TNFβ in the Cerebellum," *J Neurosci 22*(14):5813-5816, Jul. 15, 2002.

Caughey et al., "Roles of Cyclooxygenase (COX)-1 and COX-2 in Prostanoid Production by Human Endothelial Cells: Selective Up-Regulation of Prostacyclin Synthesis by COX-2," *J Immunol 167*:2831-2838, 2001.

Celotti et al., "Anti-Inflammatory Drugs: New Multitarget Compounds to Face an Old Problem. The Dual Inhibition Concept," *Pharmacological Research 43*(5):429-436, May 2001.

Chang et al., "Prevention of Lens Protein-Induced Ocular Inflammation with Cyclooxygenase and Lipoxygenase Inhibitors," *J Ocular Pharmacol 5*(4):353-360, 1989.

(56) **References Cited**

OTHER PUBLICATIONS

Chang et al., "Role of 5-Lipoxygenase Products of Arachidonic Acid in Cell-to-Cell Interaction Between Macrophages and Natural Killer Cells in Rat Spleen," *J Leukocyte Biol 50*:273-278, 1991.

Chen et al., "Oroxylin A inhibition of lipopolysacchaide-induced iNOS and COX-2 gene expression via suppression of nuclear factor-kappaB activation," *Biochemical Pharmacology 59*(11): 1445-1447, 2000.

Chen et al., "Wogonin, baicalin, and baicalein inhibition of inducible nitric oxide synthase and cyclooxygenase-2 gene expressions induced by nitric oxide syntase inhibitors and lipopolysaccharide," *Biochem. Pharmacol. 61*(11):1417-1427, 2001.

Chi et al., "Effect of wogonin, a plant flavone from *Scutellaria* radix, on the suppression of cyclooxygenase-2 and the induction of inducible nitric oxide synthase in lipopolysaccharide-treated RAW 264.7 cells." *Biochem Pharmacol 61*:1195-1203, May 15, 2001.

Chinese Herbs Direct, retrieved from http://www.chineseherbsdirect.com, 2007, 2 pages.

Chou et al., "The Antiinflammatory an Analgesic Effects of Baicalin in Carrageenan-Evoked Thermal Hyperalgesia," *Anesth Analg 97*:1724-1729, 2003.

Christie et al., "Opioids, NSAIDs and 5-lipoxygenase inhibitors act synergistically in brian via arachidonic acid metabolism," *Inflamm Res 48*:1-4, 1999.

Chung et al., "Pharmacological Effects of Methanolic Extract from the Root of *Scutellaria baicalensis* and its Flavonoids on Human Gingival Fibroblast," *Planta Med. (NY) 61*:150-153, 1995.

Clark et al., "Do Some Inhibitors of COX-2 Increase the Risk of Thromboembolic Events?," *Drug Safety 27*(7):427-456, 2004.

Commenges et al., "Intake of flavonoids and risk of dementia," *Eur. J. Epidemiol 16*:357-363, 2000.

Dafallah et al., "Investigation of the Anti-inflammatory Activity of *Acacia nilotica* and *Hibiscus sabdariffa," American Journal of Chinese Medicine 24*(3-4):263-269, 1996.

Dannhardt et al., "Cyclooxygenase inhibitors—current status and future prospects," *Eur J Med Cem 36*(2):109-126, Feb. 2001.

Davies et al., "COX-2 selective inhibitors cardiac toxicity: getting to the heart of the matter," *J Pharm Pharmaceut Sci 7*(3):332-336, 2004.

de Gaetano et al., "Prevention of thrombosis and vascular inflammation: benefits and limitations of selective or combined COX-1, COX-2 and 5-LOX inhibitors," *TRENDS in Pharmacol Sci 24*(5):245-252, May 2003.

de la Puerta et al., "Inhibition of Leukocyte Eicosanoid Generation and Radical Scavenging Activity by Gna phalin, a Lipophilic Flavonol Isolated from *Helichrysum picardii," Planta Medica 65*:507-511, 1999.

de Whalley et al., "Flavonoids Inhibit the Oxidative Modification of Low Density Lipoproteins by Macrophaes," *Biochemical Pharmacology 39*:1743-1750, 1990.

DeLange et al., "Phycoerythrin Fluorescence-Based Assay for Proxy Radicals: A Screen for Biologically Relevant Protective Agents," *Analyt Biochem 177*:300-306, 1989.

Dempke et al., "Cyclooxygenase-2: a novel target for cancer chemotherapy?," *J Cancer Res Clin Oncol 127*(7):411-417, Jul. 2001.

Deray, "Renal and cardiovascular effects of non-steroidal anti-inflammatories and selective cox 2 inhibitors," *Presse Med 33*(7):483-489, Apr. 2004. (with English abstract).

Deshpande et al., "Flavonoids of *Acacia catechu* Heartwood," *Indian Journal of Chemistry 20*:628, Jul. 1981.

DeWitt, "Cox-2-Selective Inhibitors: The New Super Aspirins," *Mol Pharmacol 55*:625-631, 1999.

Elattar et al., "Hydoxy fatty acids and prostaglandin formation in diseased human periodontal pocket tissue," *Journal of Periodontal Research 21*:169-176, 1986.

Engler et al., "The vasculoprotective effects of flavonoid-rich cocoa and chocolate," *Nutritional Res 24*:695-706, 2004.

Exotic Naturals, "*Acacia catechu* Extract," retrived Apr. 19, 2007 from, www.exoticnatural.com/acacia-catechu.htm, 2 pages.

Felson et al., "Osteoarthritis of the Knee," *The New England Journal of Medicine 354*(8):841-848, Feb. 23, 2006.

Fenton et al., "Characterization of the Effects of Antiangiogenic Agents on Tumor Pathophysiology," *Am J Clin Oncol (CCT) 24*(5):453-457, 2001.

Fiorucci et al., "Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-flammatory therapy?," *Biochem Pharmacol 62*:1433-1438, 2001.

Fosslien, "Review: Cardiovascular Complications of Non-Steroidal Anti-Inflammatory Drugs," Annals Clin. Lab. Sci. 35(4):347-370, 2005.

Fogh et al., "Modulation of Eicosanoid Formation by Lesional Skin of Psoriasis: An Ex vivo Skin Model," *Acta Derm Venerol (Stockh) 73*:191-193, 1993.

Friedman et al., "NSAIDs in Dermatologic Therapy: Review and Preview," *J Cutan Med Surg 6*(5):449-459, 2002.

Gabrielska et al., "Antioxidant Activity of Flavones from *Scutellaria baicalensis* in Lecithin Liposomes," *Verlag der Zeitschrift für Naturforschung*, pp. 817-823, 1997.

Gaffar et al., "The effect of triclosan on mediators of gingival inflammation," *J Clin Periodontal 22*:480-484, 1995.

Gafner et al., "Evaluation of the anti-inflammatory properties of skullcap (*Scutellaria lateriflora* L.) extracts in different in vitro models," 2004 International Congress on Natural Products Research, Phoenix, Arizona, Jul. 31-Aug. 4, 2004, P:60 and poster. (3 pages).

Gemma et al., "Diets Enriched in Foods with High Antioxidant Activity Reverse Age-Induced Decreases in Cerebellar β-Adrenergic Function and Increases in Proinflammatory Cytokines," *J Neurosci 22*(4):6114-6120, Jul. 15, 2002.

Genco et al., in *Contemporary Periodontics*, The C.V. Mosby Company, St. Louis, pp. 361-370, 1990.

Gilani et al., "Studies on Antihypertensive and Antispasmodic Activites of Methanol Extract of *Acacia nilotica* Pods," *Phytotherapy Res 13*(8):665-669, Dec. 1999.

Gilroy et al., "Inducible cyclooxygenase may have anti-inflammatory properties," *Nature Med 5*(6):698-701, Jun. 1999.

Giovannucci et al., "Aspirin and the Risk of Colorectal Cancer in Women," *N Engl J Med 333*(10):609-614, Sep. 7, 1995.

Goebel et al., "Procainamide, a Drug Causing Lupus Induces Prostaglandin H Synthase-2 and Formation of T Cell-Sensitizing Drug Metabolites in Mouse Macrophages," *Chem Res Toxicol 12*(6):488-500, 1999.

Gök et al., "Role of Leukotrienes on Coronary Vasconstriction in Isolated Hearts of Arthritic Rats: Effect of in vivo Treatment with CI-986, a Dual Inhibitor of Cyclooxygenase and Lipoxygenase," *Pharmacol 60*:41-46, 2000.

Gould et al., "Antioxidant protection of cerebellar β-adrenergic receptor function in aged F344 rats," *Neurosci Lett 250*:165/168, Jul. 10, 1998.

Greenspan et al., "Carboxy-Substituted Cinnamides: A Novel Series of Potent, Orally Active LTB_4 Receptor Antagonists," *J Med Chem 42*(1):164-172, 1999.

Gupta, "Flavonoids Composition or Treating Oral Disease," Third Party Observation dated May 30, 2011, for European Application No. 05810437.3, 7 pages.

Gupta, "Formulation of Dual Cyclooxygenase (COX) and Lipoxygenase (LOX) Inhibitors for Mammal Skin Care," Third Party Observation dated Jul. 2, 2011, for Canadian Application No. 2,521,429, 8 pages.

Gupta, V.K., Senior Advisor and Director, TKDL, Third Party Observations dated Oct. 9, 2010, in Canadian Application No. 02584124, 7 pages.

Hagos et al., "Isolation of Smooth Muscle Relaxing 1,3-Diary1-propan-2-ol Derivatives from *Acacia tortilis," Planta Med 53*(1):27-31, Feb. 1987.

Hamazaki et al., "Catechin's activity of inhibiting LTC4 production," *Allergy 49*(9/10):914, 2000, 2 pages. (with English translation).

Hanausek-Walaszek et al., Inhibitory Effects of Triterpenoid Saponins From *Acacia victoriae* on Dimethylbenz [A] Anthracene-Induced Murine Skin Carcinogenesis, Proceedings American Association for Cancer Research Annual Meeting 41:663 (Abstract #4216), Mar. 2000.

Haridas et al., "Avicins: Novel Triterpenoid Saponins from *Acacia victoriae* (Benth) Induce Apoptosis by Mitochondrial Perturbation,"

(56) **References Cited**

OTHER PUBLICATIONS

Proceedings American Association for Cancer Research Annual Meeting 41:361 (Abstract #3820), Mar. 2000.

Harrington et al., "Antithrombotic Therapy for Coronary Artery Disease: The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy," *Chest 126* (3 Suppl):513S-548S. Sep. 2004.

Hase et al., "Histological increase in inflammatory infiltrate in sun-exposed skin of female subjects: the possible involvement of matrix metalloproteinase-1 produced by inflammatory infiltrate on collagen degradation," *Br J Dermatol 142*(2):267-273, 2000.

Hase et al., "Peroxisome proliferator activated receptor (PPAR) dependent gene transcription activators," WPI/Thomson Database, Accession No. 2002-388616 [42], Mar. 19, 2002, 1 page.

Hennekens, "Update on Aspirin in the Treatment and the Prevention of Cardiovascular Disease," *Am J Manag Care 8*(22 suppl):S691-S700, Dec. 2002.

Heo et al., "Anti-genotoxicity of galangin as a cancer chemopreventive agent candidate," *Mutat. Res. 488*(2):135-150, 2001.

Heo et al., "Potent Inhibitory Effect of Flavonoids in *Scutellaria baicalensis* on Amyloid β Protein-Induced Neurotoxicity," *Journal of Agricultural and Food Chemistry 52*(13):4128-4132, Jun. 30, 2004. (Abstract only).

Herschman, "Regulation of prostaglandin synthase-1 and prostaglandin synthase-2," *Cancer Metastasis Rev. 13*(3-4):241-256, Dec. 1994.

Hiipakka et al., "Structure-activity relationships for inhibition human 5α-reductases by polyphenols," *Biochem Pharmacol 63*:1165-1176, 2002.

Hiraoka, "Long-Term Efficacy of COX-2 Selective Inhibitor Etodolac (Hypen®) on Chronic Low Back Pain and/or Osteoarthritis," *Clin Med 16*(7):1037(107)-1045(115), Jul. 2000, 18 pages. (English translation provided).

Hong et al., "Effects of purified green and black tea polyphenols on cyclooxygenase- and lipoxygenase-dependent metabolism of arachidonic acid in human colon mucosa and colon tumor tissues," *Biochemical Pharmacology 62*:1175-1183, 2001.

Hukkeri et al., "Anti-Inflammatory Activity of Leaves of *Acacia farnesiana* Wild," *Indian Drugs 39*(12): 664-666, Dec. 1, 2002.

Imamura et al., "Inhibitory Effects of Flavonoids on Rabit Heart Carbonyl Reductase," *J. Biochem. 127*(4):653-658, 2000.

Indian Herbal Pharmacopocia (Revised New Edition 2002), Indian Drug Manufacturers' Association, Mumbai, India, 2002, pp. 1-11.

Itoigawa et al., "Structure-activity relationship of cardiotonic flavonoids in guinea-pig papillary muscle," *J. Ethnopharmacol. 65*(3):267-272, 1999.

Itou et al., "Compsns. acting on dental caries and periodontosis—contain polyphenol cpds. pref. obtd. from tea by extn. with water," WPI/Thomson Database, Accession No. 1989-147372 [20], 1989, 1 page.

Jaeckel et al., "Correlation of Expression of Cyclooxygenase-2, Vascular Endothelial Growth Factor, and Peroxisome Proliferator-Activated Receptor δ With Head and Neck Squamous Cell Carcinoma," *Arch Otolaryngol Head Neck Surg 127*(10):1253-1259, Oct. 2001.

Jia et al., "Identification of free-B-ring flavonoids as potent cyclooxygenase 2 (COX-2) inhibitors," Derwent Abstract of U.S. Patent Application No. 2003-165588 A1, Accession No. 139:191432 CA, 1 page.

Jüni et al., "Risk of cardiovascular events and rofecoxib: cumulative meta-analysis," *Lancet 364*:2021-2029, Dec. 2004.

Kakegawa et al., "Inhibitory Effects of Tannins on Hyaluronidase Activation and on the Degranulation from rat Mesentery Mast Cells," *Chem. Pharm. Bull. 33*(11): 5079-5082, 1985.

Kalkbrenner et al., "In vitro Inhibiton and Stimulation of Purified Prostaglandin Endoperoxide Synthase by Flavonoids: Structure-Activity Relationships," *Pharmacology 44*(1):1-12, 1992.

Kaneko et al., "Protective Effect of Flavonoids on Endothelial Cells Against Linoleic Acid Hydroperoxide-induced Toxicity," *Biosci. Biotechnol. Biochem. 63*(2):323-328, 1999.

Kang et al., "Antithrombotic Activites of Green Tea Catechins and (−)-Epigallocatechin Gallate," *Thrombosis Research 96*(3):229-237, Nov. 1, 1999. (Abstract only).

Kao et al., "Modulation of Endocrine Systems and Food Intake by Green Tea Epigallocatechin Gallae," *Endocrinol 141*(3):980-987, 2000.

Kawasaki et al., "In Vitro Antiallergic Activity of Flavonoids in Histamine Release Assay Using Rat Basophilic Leukemia (RBL-2H3) Cells," *Journal of the Food Hygienic Society of Japan 33*(5): 497-503, Oct. 1994.

Khan, Mohammad Akmal Qaraabaadeen Azam wa Akmal (20th century AD), Matba Siddiqi, Delhi/Matba Mustafai, Delhi, 1909 AD p. 354, Formulation ID: AH5/199A, Formulation Name: Tila Fasaad-e-Laun, 2 pages. (with English translation).

Khan, Mohammad Akmal Qaraabaadeen Azam wa Akmal (20th century AD), Matba Siddiqi, Delhi/Matba Mustafai, Delhi, 1909 AD p. 410, Formulation ID:AH5/610, Formulation Name: Sanoon Bara-e-Zirs, 3 pages. (with English translation).

Khan, Mohammad Akmal, Qaraabaadeen Azam wa Akmal (20th century AD), Matba Siddiqi, Delhi/Matba Mustafai, Delhi, 1909 AD p. 173, Formulation ID:BA3/1032, Formulation Name: Nuskha Sanoon. (3 pages) (with English translation).

Khan, Mohammad Azam, Ikseer Azam, vol. IV (19th Century AD), p. 309, Matba Nizami, Kanpur India, 1872. (7 pages).

Khan, Mohammad Azam; Muheet-e-Azam, vol. III (19th century AD), Matba Nizami, Kanpur, 1887 AD, p. 37, Formulation ID:JA7/36D, Formulation Name: Tila Bara-e Asaraat-e-Harq, 2 pages. (with English translation).

Khan, Mohammad Najmul Ghani; Qaraabaadeen Nahm-al-Ghani (20th century AD), Munshi Nawal Kishore, Lucknow, (Second Edition), 1928 AD p. 667, Formulation ID:NA4/4357, Formulation Name: Raughan, 4 pages. (with English translation).

Kikukawa et al., "Mechanism of suppressive acton of TJ114 upon murine type II collagen-induced arthritis," *Ensho 15*(2):129-133, 1995. (abstract only).

Kim et al., "Pharmacological Activates of Flavonoids (I)—Relationships of Chemical Structure of Flavonoids and their Inhibitory Activity of Hypersensitivities," *Yakhak Hoeji 34*(5):348-364, 1990.

Kimura et al., "Effects of Baicalein Isolated from *Scutellaria baicalensis* Radix on Adhesion Molecule Expression Induced by Thrombin and Thrombin Receptor Agonist Peptide in Cultured Human Umbilical Vein Endothelial Cells," *Planta Med. 67*:331-334, 2001.

Kirchner et al., "Effects of tepoxalini, a dual inhibitor of cyclooxygenase/5-lipoxygenase, on events associated with NSAID-induced gastrointestinal inflammation," *Prostaglandins, Leukotrienes and Essential Fatty Acids 56*(6):417-423, Jun. 1997.

Kirschenbaum et al., "The Role of Cclooxygenase-2 in Prostate Cancer," *Urology 58*(Suppl 2A):127-131, Aug. 2001.

Klickstein et al., "Lipoxygenation of Arachidonic Acid as a Source of Polymorphonuclear Leukocyte Chemotactic Factors in Synovial Fluid and Tissue in Rheumatoid Arthritis and Spondyloarthritis," *J Clin Invest 66*(5):1166-1170, Nov. 1980.

Koga et al., "Effect of plasma metabolites of (+)-catechin and quercetin on monocyte adhesion to human aortic endothelial cells," *Am J Clin Nutr 73*:941-948, 2001.

Kong, "Aspirin in Cardiovascular Disorders—What is Optimum Dose?," *Am J Cardiovasc Drugs 4*(3):151-158, 2004.

Krakauer et al., "The flavonoid baicalin inhibits superantigen induced inflammatory cytokines and chemokines," *FEBS Lett. 500*:52-55, 2001.

Kubo et al., "Flavonols from *Heterotheca inuloides*: Tyrosinase Inhibitory Activity and Structural Criteria," *Bioorg. Med. Chem. 8*(7):1749-1755, 2000.

Kubo et al., "Studies on Scutellariae Radix. Part II: The Antibacterial Substance," *Journal of Medicinal Plant Research 43*:194-201, 1981.

Kubo et al., "Studies on *Scutellariae* Radix, VII. Anti-Arthritic and Anti-inflammatory Actions of Methanolic Extract and Flavonoid Components from *Scutellariae* Radix," *Chem. Pharm. Bull, 32*(7):2724-2729, 1984.

(56) **References Cited**

OTHER PUBLICATIONS

Kuhn et al., "Action of cyclooxygenase (COX) and lipoxygenase (LOX) inhibitors as well as of oxygen free radical scavengers (OFRS) in the inflammation-induced vasodepression," *Biomed Biochim Acta* 47(10/11):S320-S323, 1988.

Kulkarni et al., "Licofelone—A Novel Analgesic and Anti-Inflammatory Agent," *Current Topics Med Chem* 7(3):251-263, 2007.

Kuppusamy et al., "Effects of Flavonoids on Cyclic Amp Phosphodiesterase and Lipid Mobilization in Rat Adipocytes," *Biochem Pharmacol* 44(7):1307-1315, 1992.

Kuppusamy et al., "Potentiation of β-Adrenoceptor Agonist-Mediated Lipolysis by Quercetin and Fisetin in Isolated Rat Adipocytes," *Biochem Pharmacol* 47(3):521-529, 1994.

Lamarque, "Safety of the selective inhibitors the inducible cyclooxygenase-2 taken for long period," *Bull Cancer* 91:S117-S124, 2004.

Laughton et al., "Inhibiton of Mammalian 5-Lipoxygenase and Cyclo-Oxygenase by Flavonoids and Phenolic Dietary Additives," *Biochem Phamacol* 42(9):1673-1681, 1991.

Lee et al., "Inhibition of oxidative DNA damage, 8-OHdG, and carbonyl contents in smokers treated with antioxidants (vitamin E, vitamin C, β-carotene and red ginseng)," *Cancer Lett* 132:219-227, 1998.

Lee et al., "Pharmacokinetics of Tea Catechins after Ingestion of Green Tea and (—)-Epiallocatechin-3-gallate by Humans: Formation of Different Metabolites and Individual Variability," *Cancer Epidemiol, Biomarkers & Prev* 11:1025-1032, Oct. 2002.

Lee et al., "Salicylic Acid Peels for the Treatment of Acne Vulgaris in Asian Patients," *Dermatol Surg* 29(12):1196-1199, 2003.

Lenton et al., "Ability of human plasma to protect agianst ionizing radiation is inversely correlated with age," *Mech Ageing Dev* 107:15-20, Feb. 1, 1999.

Levy et al., "Safety and efficacy of flavocoxid compared with naproxen in subjects with osteoarthritis of the knee: a pilot study," *ICRS 2007*, 5 pages.

Leyden, "A review of the use of combination therapies for the treatment of acne vulgaris," *J Am Acad Dermatol* 49(3 Suppl):S200-S210, 2003. (Abstract only).

Leyden, "Guest Editorial," *J Am Acad Dermatol* 49(3 Suppl.):S199, 2003.

Li et al., "Chemoprevention of 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamster check pouch by a cyclooxygenase 2 inhibitor (Celecoxib) and a 5-lipoxygenase inhibitor (Zileuton)," *AACR Meeting Abstracts*, Abstract No. 546-a, 2004. (1 page).

Li et al., "The effect of Radix *Scutellariae* on butyrate of *Porphyromonas endoantalis* in vitro," West China J. Stomatol. 22(1):57-61, 2004, 1 page. (Abstract only).

Li et al., "The Flavonoid baicalin exhibits anti-inflammatory activity by binding to chemokines," *Immunopharmacology* 49:295-306, 2000.

Liang et al., "Suppression of inducible cyclooxygenase and nitric oxide synthase through activation of peroxisome proliferator-activaed receptor-γ by flavonoids in mouse macrophages," *FEBS Lett.* 496(1):12-18, 2001.

Liao et al., "Selective Inhibition of Steroid 5α-Reductase Isozymes by Tea Epicatechin-3-Gallate and Epigallaocatechin-3-Gallate," *Biochem Biophys Res Comm* 214(3):833-838, 1995.

Lotioncrafter.com, "PEG-7 Glyceryl Cocoate," retrieved Apr. 17, 2008, from http://www.lotioncrefter.com/store/PEG-7-Glyceryl-Cocoate-pr-16444.html, 1 page.

Lubrizol, Material Safety Data Sheet: CARBOPOL * ULTREZ 21 Polymer, effective date Oct. 17, 2007, 8 pages.

Lynch, "Age-Related Impairment in Long-Term Potentiation in Hippocampus: A Role for the Cytokine, Interleukin-1β?," *Prog Neurobiol* 56:571-589, Dec. 1998.

Mādhavah; Vrndamādhava;—Marathi translated by Datto vallala Borkar; Yagyeswar Gopal Dixit, Bookseller, Pune; Ed. 1922 [Time of origin 9th century], p. 503, Formulation ID:AB/1051, Formulation Name:Khadirodakam, 3 pages. (with English translation).

Marjoosi, Ai Lon-e-Abbaas, Kaamil-al-Sena'ah, Part II (10th century AD), Central Council for Research in Unani Medicine, 61-85 Institutional Area, Janak Puri, New Delhi-58, 2005, p. 129; Formulation ID:Ah3/876C; Formulation Name:Zimaad Baraae Qooba, 3 pages. (with English Translation).

Matsumoto et al., "Concordant Induction of Prostaglandin E_2 Synthase with Cyclooxygenase-2 Leads to Preferred Production of Prostaglandin E_2 over Thromboxane and Prostaglandin D_2 in Lipopolysaccharide-Stimulated Rat Peritoneal Macrophages," *Biochem Biophys Res Commun* 230(1): 110-114, 1997.

Mayo Clinic, "Alzheimer's disease," Feb. 10, 2009, 2 pages.

McAdam et al., "Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: The human pharmacology of a selective inhibitor of COX-2" *Proc. Natl. Acad. Sci. USA* 96:272-277, Jan. 1999.

MedicineNet.com "Rheumatoid Arthritis (RA)," Retrieved on Jun. 4, 2009 from, www.medicinenet.com/rheumatiod_arthritis/article.htm, 4 pages.

Meyer et al., "Antiviral of galangin isolated from the aerial parts of *Helichrysum aureonitens*," *J. Ethnopharmacol.* 56(2):165-169, 1997.

Millikan, "The Rationale for Using a Topical Retinoid for Inflammatory Acne," *Am J Clin Dermatol* 4(2):75-80, 2003.

Min et al., "(-)-Epiafzelechin: Cyclooxygenase-1 Inhibitor and Anti-Inflammatory Agent from Aerial Parts of *Celastrus orbiculatus*," *Planta Med.* 65:460-462, 1999.

Miyamoto et al., "Studies on selection method of crude drugs by statistical analysis. Research on Rhubarb having anti-inflammatory activity," *Natural Medicine* 55(4):159-164, 2001. (with English abstract).

Montine et al., "Antioxidants significantly affect the formation of different classes of isoprostanes and neuroprostanes in rat cerebral synaptosomes," *Biochem Pharmacol* 65(4):611-617, Feb. 15, 2003.

Moore et al., "COX-2 Inhibition, Apoptosis, and Chemoprevention by Nonsteroidal Anti-inflammatory Drugs," *Curr Med Chem* 7(11):1131-1144, 2000.

Morimoto et al., "Effects of *Bofu-tsusho-san*, a traditional Chinese medicine on body fat accumulation in fructose-loaded rats," *Nippon Yakurigaky Zasshi* 117:77-86, 2001. (English Abstract provided).

Moroney et al., "Selectivity of Neutrophil 5-Liposygenase and Cyclo-oxygenase Inhibition by an Anti-inflammatory Flavonoid Glycoside and Related Aglycne Flavonoids," *J. Pharm. Pharmacol.* 40:787-792, 1988.

Murari et al., "A Study of the Components of Cutch: Isolation of Catechin, Gallocatechin, Dicateechin & Catechin Tetramer as Methyl Ethers," *Indian Journal of Chemistry 14B*(9):661-664, 1976.

Murase et al., "Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver," *International Journal of Obesity* 26:1459-1464, 2002.

Murray et al., "Dietary Supplementation with Vitamin E Reverses the Age-related Deficit in Long Term Potentiation in Dentate Gyrus," *J Biol Chem* 273(20):1261-12168, May 15, 1998.

Mutoh et al., "Suppression by Flavonoids of Cyclooxygenase-2 Promoter-dependent Transcriptional Activity in Colon Cancer Cells: Structure-Activity Relationship," *Jnp. J. Cancer Res.* 91:686-691, 2000.

Nadkarni, ed., *Dr. K.M. Nadkarni's Indian Materia Medica: With Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic & Home Remedies, Appendices & Indexes 1*:8-17, Popular Prakashan, London, 1996.

Nagai et al., "Inhibition of Mouse Liver Sialidase by the Root of *Scutellaria baicalensis*," *Planta Medica* 55:27-29, 1989.

Nakagami (Aug. 22, 1995) abstract DATABASE WPI Week 199519 Aug. 22, 1995, Derwent Publications Ltd., London GB; Class 954, p. 2, AN 1995-325471 XP002418722 "Anti-complementary substance used as therapeutic agent—comprises gallic acid, methyl gallate, acetyl-salicylic acid, caffeic acid, catechin, epi-gallo-catechin gallate, myricetin, quercitrin and/or baicalein, or their salts," & JP07 223941 A ((NIHA-N) NIPPON HAM KK).

Nakahata et al., "Analysis of Inhibitory Effects of *Scutellariae* Radix and Baicalein on Prostaglandin E2 Production in Rat c6 Glimo Cells," *Am. J. Chin. Med.* 26311-323, 1998.

(56) **References Cited**

OTHER PUBLICATIONS

Nakahata et al., "Mitogen-activated Protein Kinase," *Nippon Yakurigaku Zasshi 114*(11):215P-219P, 1999.

Nakajima et al., "Inhibitory Effect of Baicalein, a Flavonoid in *Scutellaria* Root, on Eotaxin Production by Human Dermal Fibroblasts," *Planta Med. 67*(2):132-135, 2001.

Nakamura et al., "Arachidonic Acid Cascade Inhibitors Modulate Phorbol Ester-Induced Oxidative Stress in Female ICR Mouse Skin; Differential Roles of 5-Lipoxygenase and Cyclooxygenase-2 in Leukoeyte Infiltration an Activation," *Free Radical Biol Med 35*(9):997-1007, Aug. 2003.

Nityanāthasiddhah; Rasartnākarah-Rasendra khandam Comm. Datto VallāBorakara, Ed. 2nd, 1986, Shri Gajānan Book Depot, (Pune), p. 756, Formulation ID: RJ/30, Formulation Name: Kusthaharalepah (02), 3 pages. (with English translation).

Niwa et al., "Applicant of New Drugs to the Elderly (12)—COX-2 Selective Inhibitor," *Geriatric Gastroenterology 10*(3):181-184, 1998, (with English translation).

Noreen et al., "Development of a Radiochemical Cyclooxygenase-1 and -2 in Vitro Assay for Identification of Natural Products as Inhibitors of Prostaglandin Biosynthesis," *Journal of Natural Products 61*:2-7, Jan. 1998

Noreen et al., "Flavan-3-ols Isolated From Some Medicinal Plants Inhibiting COX-1 and COX-2 Catalyzed Prostaglandin Biosynthesis," *Planta Medica 64*:520-524, 1998.

Noreen et al., "Two New Isoflavones from *Ceiba pentandra* and Their Effect on Cyclooxygenase-Catalyzed Prostaglandin Biosynthesis," *Journal of Natural Products 61*:8-12, Jan. 1998.

Nuratracon 2008 NutriAward, retrieved May 1, 2008, from http://www.nutraconference.com/nutraaward.index.cfm, 2 pages.

Office Action issued Apr. 13, 2007, for U.S. Appl. No. 10/462,030, 15 pages.

Office Action Issued Aug. 7, 2007, for U.S. Appl. No. 10/817,330, 11 pages.

Office Action Issued Dec. 21, 2007, for U.S. Appl. No. 10/462,030, 7 pages.

Office Action issued Feb. 13, 2009, for U.S. Appl. No. 11/962,363, 13 pages.

Office Action issued Oct. 30, 2008, for U.S. Appl. No. 10/427,746, 12 pages.

Office Action Issued Feb. 20, 2008, for U.S. Appl. No. 10/817,330, 11 pages.

Office Action issued Feb. 26, 2009, for U.S. Appl. No. 11/661,382, 15 pages.

Office Action issued Feb. 8, 2010, for U.S. Appl. No. 11/279,925, 8 pages.

Office Action Issued Feb. 9, 2007, for U.S. Appl. No. 10/817,330, 20 pages.

Office Action issued Jan. 10, 2007, for U.S. Appl. No. 10/932,571, 8 pages.

Office Action issued Jan. 2, 2009, for U.S. Appl. No. 11/927,061, 17 pages.

Office Action issued Jan. 4, 2007, for U.S. Appl. No. 10/427,746, 10 pages.

Office Action Issued Jul. 15, 2008, for U.S. Appl. No. 10/462,030, 9 pages.

Office Action issued Jul. 19, 2006, for U.S. Appl. No. 10/427,746, 6 pages.

Office Action issued Jun. 15, 2009, for U.S. Appl. No. 11/279,925, 27 pages.

Office Action issued Jun. 27, 2007, for U.S. Appl. No. 10/932,571, 9 pages.

Office Action issued Mar. 21, 2008, for U.S. Appl. No. 10/427,746, 9 pages.

Office Action Issued Mar. 5, 2009, for U.S. Appl. No. 10/462,030, 8 pages.

Office Action issued Sep. 21, 2007, for U.S. Appl. No. 10/427,746, 12 pages.

Office Action Issued Sep. 3, 2009, for U.S. Appl. No. 10/817,330, 9 pages.

Oringer, "Modulation of the Host Response in Periodontal Therapy," *J Periodontal 73*(4):460-470, Apr. 2002.

Parente, "Pros and Cons of Selective Inhibition of Cyclooxygenase-2 versus Dual Lipoxygenase/Cyclooxygenase Inhibition: Is Two Better than One?," *J Rheumatol 28*:2375-2382, Nov. 2001.

Park et al., "Involvement of ERK and Protein Tyrosine Phosphatase Signaling Pathways in EGCG-Induced Cyclooxygenase-2 Expression in Raw 264.7 Cells," *Biochemical and Biophysical Research Communications 286*:721-725, 2001.

Patrono et al., "DRUG THERAPY: Low-Dose Aspirin for the Prevention of Atherothrombosis," *N. Engl. J. Med. 353*(22):2373-2383, 2005.

Phillips et al., "Polarized light photography enhances visualization of inflammatory lesions of acne vulgaris," *J Am Acad Dermatol 37*(6):948-952, 1997.

Rae et al., "Leukotriene B_4 An Inflammatory Mediator in Gout," *Lancet 320*(8308):1122-1124, Nov. 1982.

Rainsford, "The ever-emerging anti-inflammatories. Have there been any real advances?," *J Physiol—Paris 95*:11-19, 2001.

Ramesiiwaii et al., "Chemical Constituents of *Acacia*," 1997, 2 pages.

Raso et al., "Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in microphage J774A," *Life Sci. 68*(8):921-931, 2001.

Raz et al., "Differential modification of cyclo-oxygenase and peroxidase activities of prostaglandin endoperoxidase synthase by proteolytic digestion and hydroperoxides," *Biochem J 269*(3):603-607, 1990.

Raz et al., "Regulation of Fibroblast Cyclooxygenase Synthesis by Interleukin-1," *J Biol Chem 263*(6):3022-3028, Feb. 25, 1988.

Rioja et al., "An anti-inflammatory ditriazine inhibiting leukocyte functions and expression of inducible nitric oxide synthase and cyclo-oxygenase-2," *Eur J Pharmacol 397*(1):207-217, May 2000.

Rocca et al., "Cyclooxygenase-2 expression is induced during human megakaryopoiesis and characterizes newly formed platelets," *PNAS USA 99*(11):7634-7639, May 2002.

Römpp Encyclopedia Natural Products eds. W. Steglich, B. Fugmann and S. Lang-Fugmann, Georg Thieme Verlag, Stuttgart, Germany, 2000, p. 630, 3 pages.

Saleem et al., "Chemistry of the Medicinal Plants of Genus *Acacia*," *Hamdard Medicus 41*(1):63-67, 1998.

Salmon et al., "Evaluation of Inhibitors of Eicosanoid Synthesis in leukocytes: Possible Pitfall of Using the Calcium Ionophore A23187 to Stimulate 5' Lipoxygenase," *Prostaglandins 29*(3): 377-385, 1985.

Sartor et al., "Inhibition of matrix-proteases by polyphenols: chemical insights for anti-inflammatory and anti-invasion drugs design," *Biochem Pharmacol 64*:229-237, 2002.

Sekine et al., "Structure and Synthesis of a New Monoterpenoidal Carboxamide from the Seeds the Thai Medicinal Plant *Acacia concinna*," *Chemistry and Pharmaceutical Bulletin 45*(1):148-151, 1997.

Shah et al., "The Antiplatelet Aggregatory Activity of *Acacia nilotica* is Due to Blockade of Calcium Influx through Membrane Calcium Channels," *General Pharmacology 29*(2):251-255, 1997.

Sharma, "Chemical Constituents of *Acacia catechu* Leaves," *J Indian Chem Soc 74*, Jan. 1997.

Sharon et al., "Production of Leukotriences by Colonie Mucosa from Patients with Inflammatory Bowel Disease (IBD)," *Gastroenterology 84*(5), 1983.

Shen, "Inhibition of thrombin: relevance to anti-thrombosis strategy," *Frontiers Biosci 11*:113-120, Jan. 2006.

Shibata et al., "Pharmacological Study of Kumazasa (Report 1) Acute toxicity, anti-inflammatory and antiulcerative effects of kumazasa water soluble fraction (Folin)," Folia pharmacol Japan 71(5):481-490, 1975.

Smalley et al., "Use of Nonsteroidal Anti-inflammatory Drugs and Incidence of Colorectal Cancer," *Arch Intern Med 159*(2):161-166, Jan. 25, 1999.

(56) **References Cited**

OTHER PUBLICATIONS

SmartSkinCare.com, "What green tea can and cannot for your skin," retrieved Jun. 5, 2009 from www.smartskincare.com/treatments/topical/greentea.html, 3 pages.

So et al., "Inhibition of proliferation of estrogen receptor-positive MCF-7 human breast cancer cells by flavonoids in the presence and absence of excess estrogen," *Cancer Lett. 112*(2):127-133, 1997.

Sobottka et al., "Effect of Flavonol Derivatives on the Carrageenin-Induced Paw Edema in the Rat and Inhibition of Cyclooxygenase-1 and 5-Lipoxygenase in Vitro," *Arch. Pharm. Med. Chem. 333*:205-210, 2000.

Sodhala; Gadanigrahah ed, Ganga Sahayah Pandeya & Com., Indradeva Tripathi, Part-1(Prayoga Khanda) Chaukhamba Sanskrit Sansthan, Varanasi, Ed. 3rd 1999, p. 107-108, Formulation ID:AK2/158, Formulation Name: Khadiradyam Tailam, 6 pages. (with English translation).

Sodhala; Gadanigrahah ed, Ganga Sahayah Pandeya & Com., Indradeva Tripathi, Part-3(Salakya-Pancakarma Khanda) Chaukhamba Sanskrit Sansthan(Varanasi) Ed. 3rd 1999, p. 225, Formulation ID: RG2/525, Formulation Name: Dantasula Cikitsa, 3 pages. (with English translation).

Stadtman et al., "Reactive Oxygen-Mediated Protein Oxidation in Aging and Disease," *Drug Metab Rev 30*:225-243, May 1998.

Stanke-Labesque et al., "Angiotensin II-induced contractions in human internal mammary artery: effects of cycloxygenase and lipoxygenase inhibition," *Cardiovasc Res 47*:376-383, 2000.

Sugiyama, "The roots of Cha and Gambir," *Yakushigaku Zasshi 40*(2):98-106, 2005.

Takada, "Catechin-containing soap includes tea-oriented catechin as one of green tea ingredients," WPI/Thomson Database, Accession No. 1999-367373 [31], Oct. 31, 1997, 1 page.

Tanaka, "Cosmetics for preventing aging of skin, comprises elastase inhibitor such as catechin, flavones, flavonols, flavanone, isoflavanones, coumarin and/or their glycosides" WPI/Thomson Database, Acession No. 2003-451711 [43], & JP 2003002820A, Jan. 8, 2003, 4 pages.

Tordera et al., "Influence of Anti-Inflammatory Flavonoids on Degranulation and Arachidonic Acid Release in Rat Neutrophils," *Z. Naturforsch* [c] *49*:235-240, 1994.

Tsao et al., "Effect of Chinese and Western Antimicrobial Agents on Selected Oral Bacteria," *Journal of Dental Research 61*(9):1103-1106, 1982.

Vāgbhata; Astānga Samgraha—(commentary by Indu), Part-I(KA); Central Council for Research in Ayurveda &Siddha, New Delhi, 1991, Time of origin 5-10th century, p. 27, Formulation ID: AT/2103, Formulation Name: Gandūsadhāranādigunaāh, 3 pages, (with translation).

Vautrin, "Etude botanique chimique et pharmacologique do genre *Acacia*. (Botanical, chemical and pharmacological study of the *Acacia* species).," Universitie de Dijon (France) 94pp., 1996, Abstract #58/646c in *Dissertation Abstracts International 58*(1):177-C, 1997.

Verheugr, "New anticoagulants in ischemic heart disease," *Presse Med. 34*:1325-1329, 2005.

Wakabayashi et al., "Wogonin inhibits inducible prostaglandin E2 production in macrophages," *Eur. J. Pharmacol. 406*(3):477-448, 2000.

Wakdikar, "Global health care challege; Indian experiences and new prescriptions," *Electronic Journal of Biotechnology 7*(3):217-223, Dec. 15, 2004.

Wallace et al., "Limited anti-inflammatory efficacy of cyclo-oxygenase-2 inhibition in carrageenan-airpouch inflammation," *Br J Pharmacol 126*:1200-1204, 1999.

Wang, "Cyclooxygenase active bioflavonoids from Balaton™ tart cherry and their structure ativity relationships," *Phytomedicine 7*:15-19, 2000.

Weinberg, "Nitric Oxide Synthase 2 and Cyclooxygenase 2 Interactions in Inflammation," *Immunol Res 22*(2-3):319-341, 2000.

Wenzel et al., "Dietary Flavone is a Potent Apoptosis Inducer in Human Colon Carcinoma Cells," *Cancer Res. 60*:3823-3831, 2000.

Whelton et al., "Nonsteroidal Anti-Inflammatory Drugs: Effects on Kidney Function," *J Clin Pharmacol 31*:588-598, 1991.

Whiteman et al., "Protection Against Peroxynitrite-Dependent Tyrosine Nitration and α_1-Antiproteinase Inactivation by Ascorbic Acid. A Comparison with other Biological Antioxidants," *Free Rad Res 25*(3):275-283, Sep. 1996.

Wilgus et al., "Inhibition of Ultraviolet Light B-Induced Cutaneous Inflammation by a Specific Cyclooxygenase-2 Inhibitor," *Adv Exp Med Biol 507*:85-92, 2002.

Wilgus et al., "Topical application of a selective cyclooxygenase inhibitor suppresses UVB mediated cutaneous inflammation," *Prostaglandins & other Lipid Mediators 62*(4):367-384, 2000.

Winter, "Unigen's Univestin Targets joint inflammation," Func. Foods Nutraceut, p. 14, May 2005.

Wollheim, "Approaches to rheumatoid arthritis in 2000," *Curr Opin Rheumatol 13*(3):193-201, 2001.

Xiaozhen et al., "Induction of PGE_2 Production and COX-2 Expression in Human Gingival Fibroblasts Stimulated with LPS," *Med J Wuhan Uni 23*(4): 301-305, Oct. 2002. (with English Abstract).

Xie et al., "Mitogen-Inducible Prostaglandin G/H Synthase: A New Target for Nonsteroidal Antiinflammatory Drugs," *Drug Development Research 25*(4):249-265, 1992.

Xu et al., "[Stduy on flavonoids in *Ligustrum lucidum*]," *Zhong Yao Cai 30*(5):538-540, May 2007, 1 page. (Abstract Only).

Yamahara et al., "Inhibitory effect of crude Chinese drugs on the denaturation of human γ-globulin induced by heat and copper (2+)," *Shoyakugaku Zasshi 35*(2): 103-107, 1981.

Yang et al., "Effects of Green Tea Catechin on Phospholipase A_2 Activity and Antithrombus in Streptozotocin Diabetic Rats," *J Nutr Sci Vitaminol 45*:337-346, Jun. 1999.

Ye et al., "Anticancer Activity of *Scutellaria baicalensis* and its Potential Mechanism," *The Journal of Alternative and Complementary Medicine 8*(5):567-572, 2002.

Yoshida et al., "Thermogenic, anti-obesity effects of *bofu-tsusho-san* in MSG-obese mice," *International J Obesity 19*:717-722, 1995.

You et al., "Cyclooxygenase/Lipoxygensae from Human Platelets by Polyhydroxlated/Methoxylated Flavonoids Isolated from Medicinal Plants," *Arch. Pharm. Res. 22*(1):18-24, 1999.

Young et al., "The Mouse Ear Inflammatory Response to Topical Arachidonic Acid," *J Invest Dermatol 82*(4):367-371, 1984.

Zhang et al., "Inhibition of Cancer Cell Proliferation and Prostaglandin E_2 Synthesis by *Scutellaria baicalensis," Cancer Research 63*:4037-4043, 2003.

Zhang et al., *China Journal of the Chinese Materia Medical 27*(4):254-257, 2002. (English translation provided).

Arzani, Mohammad Akbar, "Qaraabaadeen Qaadri," MH5/249 Zimaad-e-Shaqeeqaa, *Ahmadi Publication*, p. 45, Delhi, India, 1968, 8 pages, (with English Translation).

Bharata Bhalsajya Ratnakara, vol. III, compiled by N.C. Saha, 2nd Ed. Reprint, p. 481, B. Jain Publishers, New Delhi, India, 1999, 11 pages. (with English Translation).

Bhatta, "Siddhabhesajamanimala," Chaukhamba Krishnadas Academy 3rd Ed., Varanasi, India, p. 237, 2003, 8 pages.

Gupta, Third Party Submission, dated Feb. 15, 2013; received in USPTO Feb. 25, 2013.

Kaiyadeva, Kaiyadevanighantau—(Pathyāpthyavibodhakah), 1st Ed., P.V. Sharma and G.P. Sharma, Eds., p. 153, Chaukhambha Orientalia, Varamasi, India, 1979, (8 pages) (with English Translation).

Reinhard, "*Uncaria tomentosa* (Willd.) D.C.: Cat's Claw, Uña de Gato, Savéntaro," *The Journal of Alternative and Complementary Medicine 5*(2):143-151, 1999.

Suśruta, Suśruta Samhitā(commentary by Dalhanna), vol. II 1st Ed., P.V Sharma, Ed., pp. 461-462, Chaukhambha Visvabharati, Varanasi, India, 2000. (9 pages) (with English Translation).

Vāgbhata, Astānga Hrdaya (commentaries by Arunadatta and Hemādri), 8th Ed., B. H. P. Vaidya, Ed., p. 715, Chaukhambha Orientalia, Varanasi, India, 1998, (9 pages) (with English translation).

(56) **References Cited**

OTHER PUBLICATIONS

Brhat Nighantu Ratnākara (Saligramanighantubhusanam) Translated by Sri Dattarama Srikrsnalala Mathura; vol. 4 (Part VII)—Compiled by Gaṅgāvisnu Śrīkrsna Dāsa, Page(s) being submitted—05 (p. 04-08), (Ref. p. no. of publication:506), edn. 1997, Khemaraja Srikrsnadas Prakasana, Mumbai-4, India.†

Mohammad Akmal Khan, Qaraabaadeen Azam wa Akmal (19th century AD), Page(s) being submitted—04 (p. 09-12), (Ref. p. no. of publication:514), 1909 AD, Matba Siddiqi / Matba Mustafai, Delhi, India.†

Mohammad Shareef Khan, Ilaaj-al-Amraaz, Page(s) being submitted—06 (p. 13-17), (Ref. p. no. of publication:304), 1921, Afzal-al-Matabe, Barqi Press, Delhi, India.†

† cited by third party

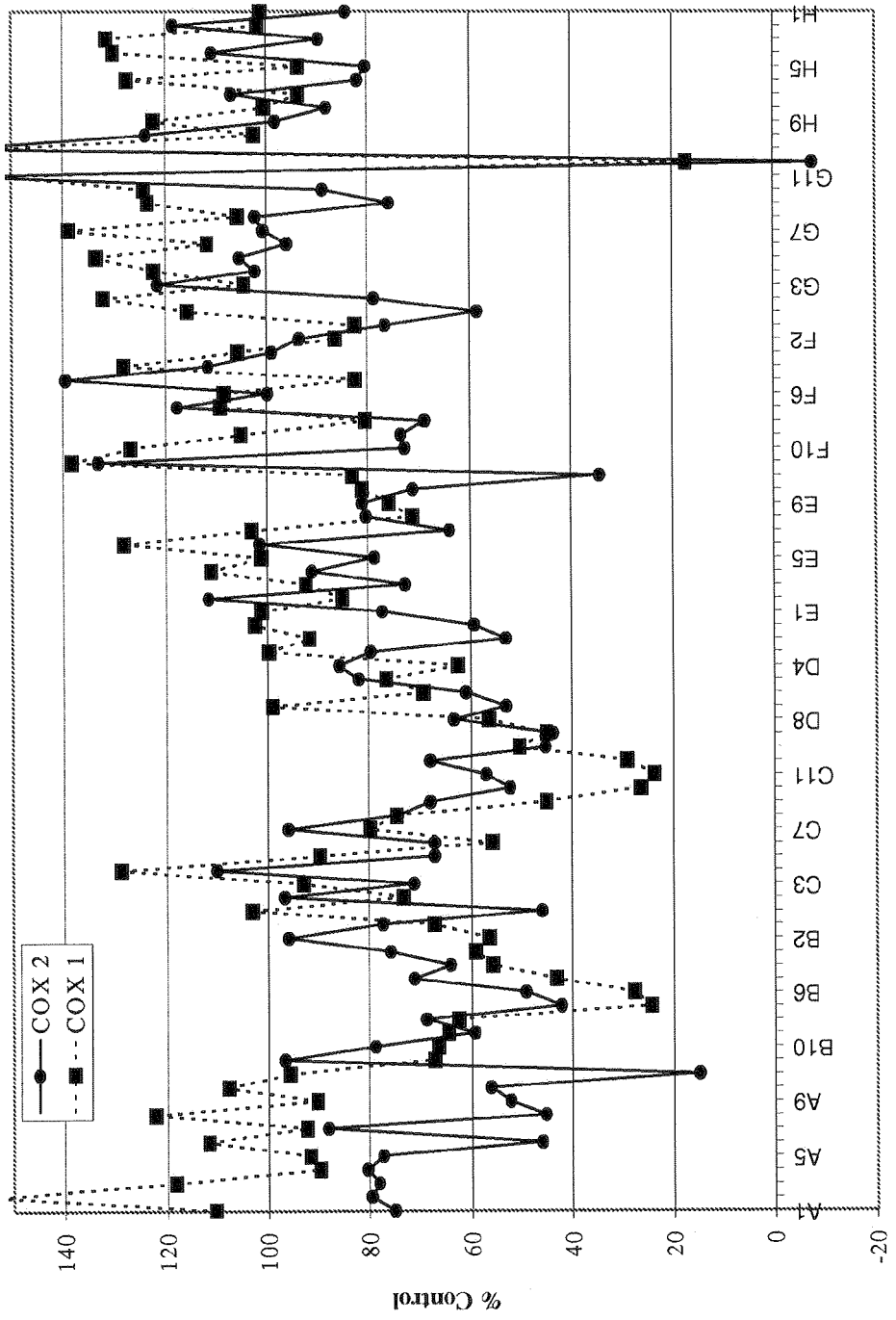

COX 2
COX 1
% Control
140
120
100
80
60
40
20
0
-20
A1
A5
A9
B10
B6
B2
C3
C7
C11
D8
D4
E1
E5
E9
F10
F6
F2
G3
G7
G11
H9
H5
H1
Well Number

Figure 1

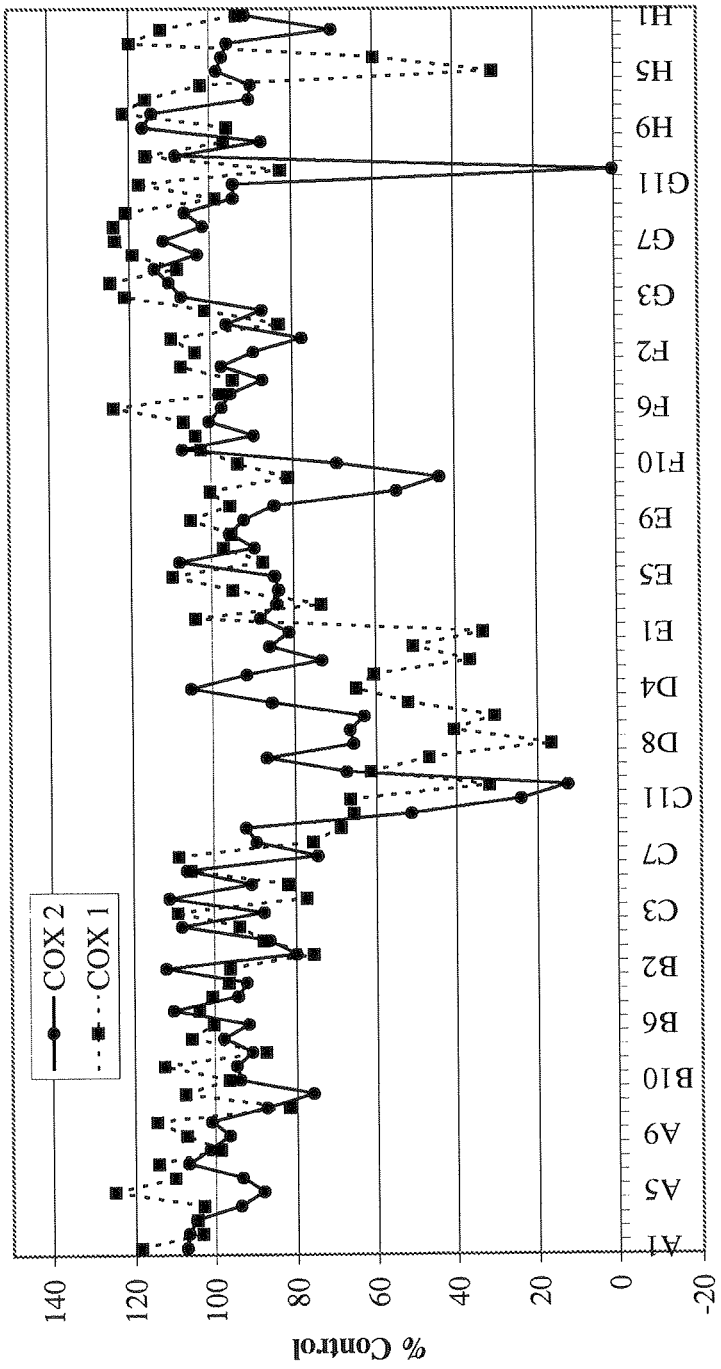

COX 2
COX 1
% Control
140
120
100
80
60
40
20
0
-20
A1
A5
A9
B10
B6
B2
C3
C7
C11
D8
D4
E1
E5
E9
F10
F6
F2
G3
G7
G11
H9
H5
H1
Well Number

Figure 2

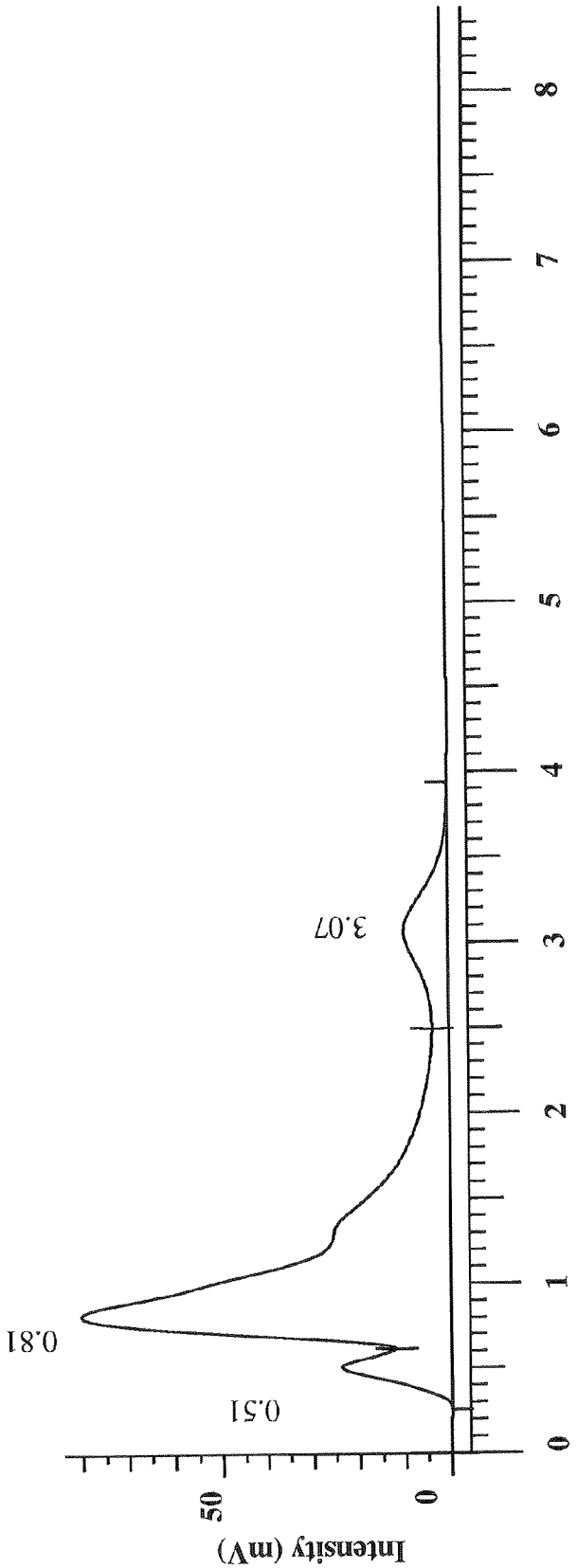


Figure 3A

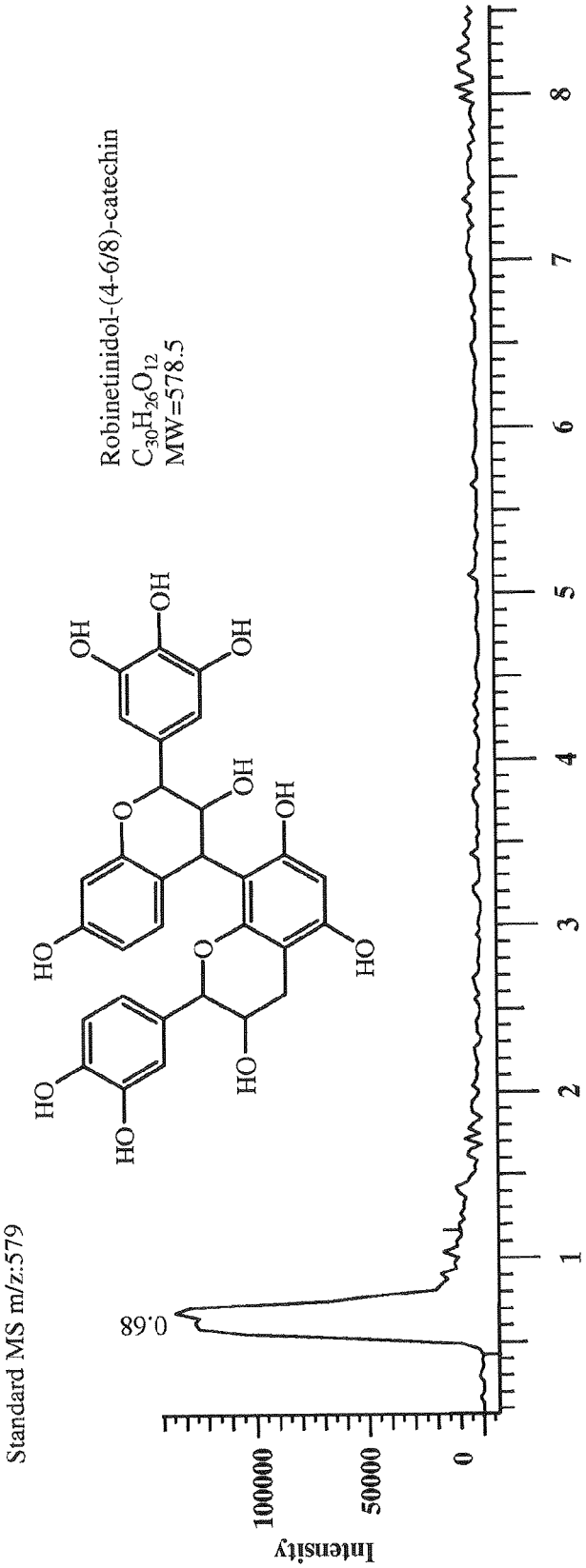

Standard MS m/z:579
Robinetinidol-(4-6/8)-catechin
$C_{30}H_{26}O_{12}$
MW=578.5
Intensity
100000
50000
0
0.68
1
2
3
4
5
6
7
8
Retention Time (min)

Figure 3B

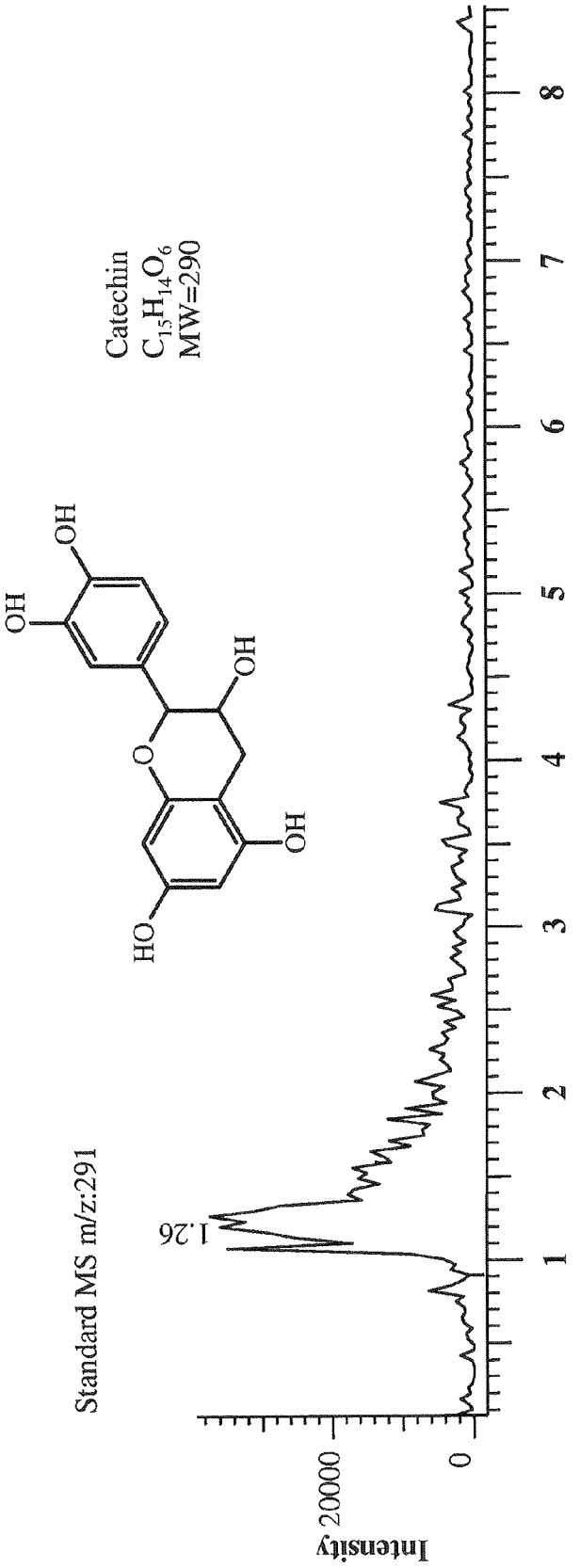

Standard MS m/z:291
Catechin
$C_{15}H_{14}O_6$
MW=290
OH
OH
OH
OH
HO
O
1.26
Intensity
20000
0
1
2
3
4
5
6
7
8
Retention Time (min)

Figure 3 C

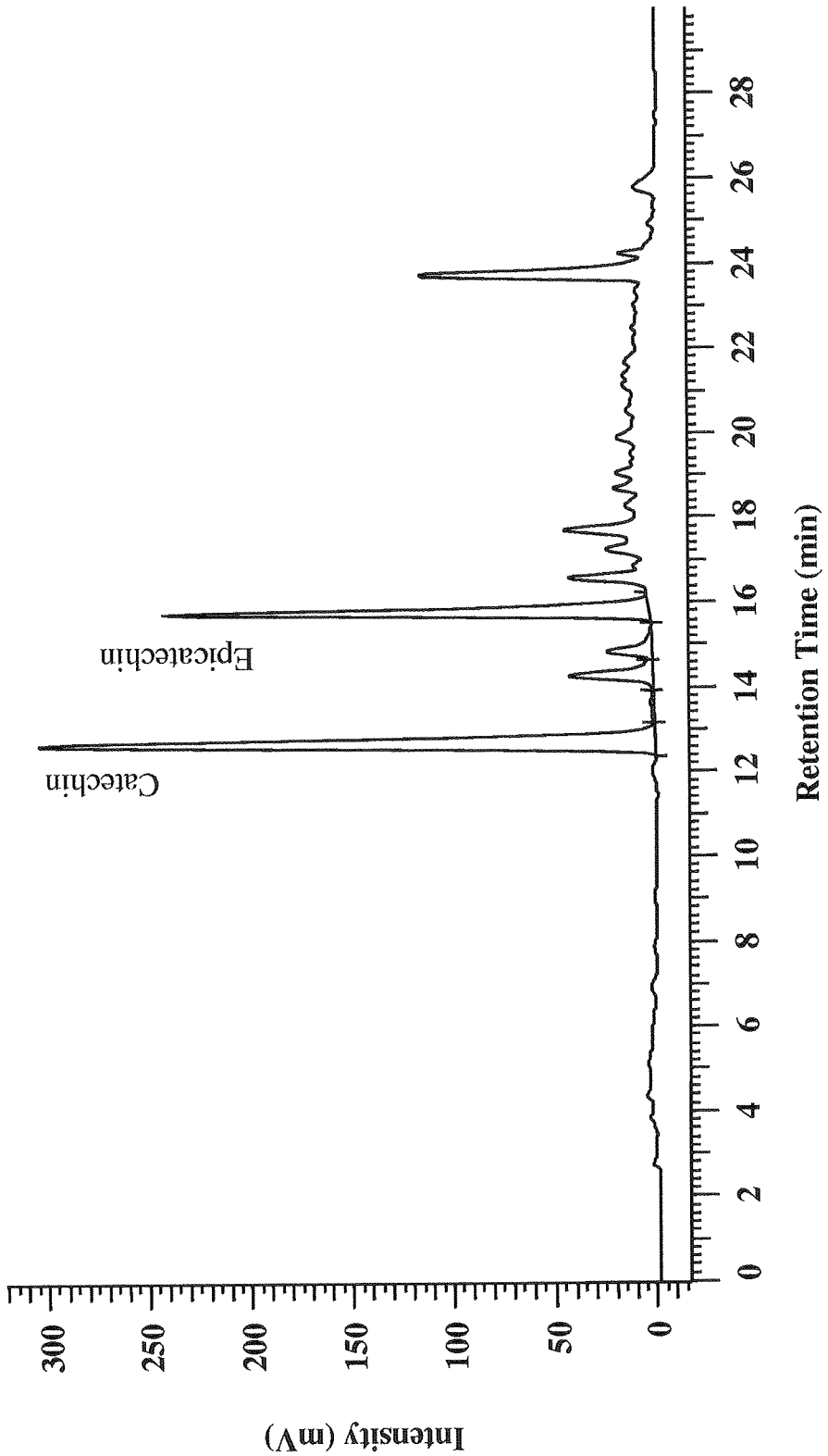

Intensity (mV)
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Retention Time (min)
Catechin
Epicatechin

Figure 4A

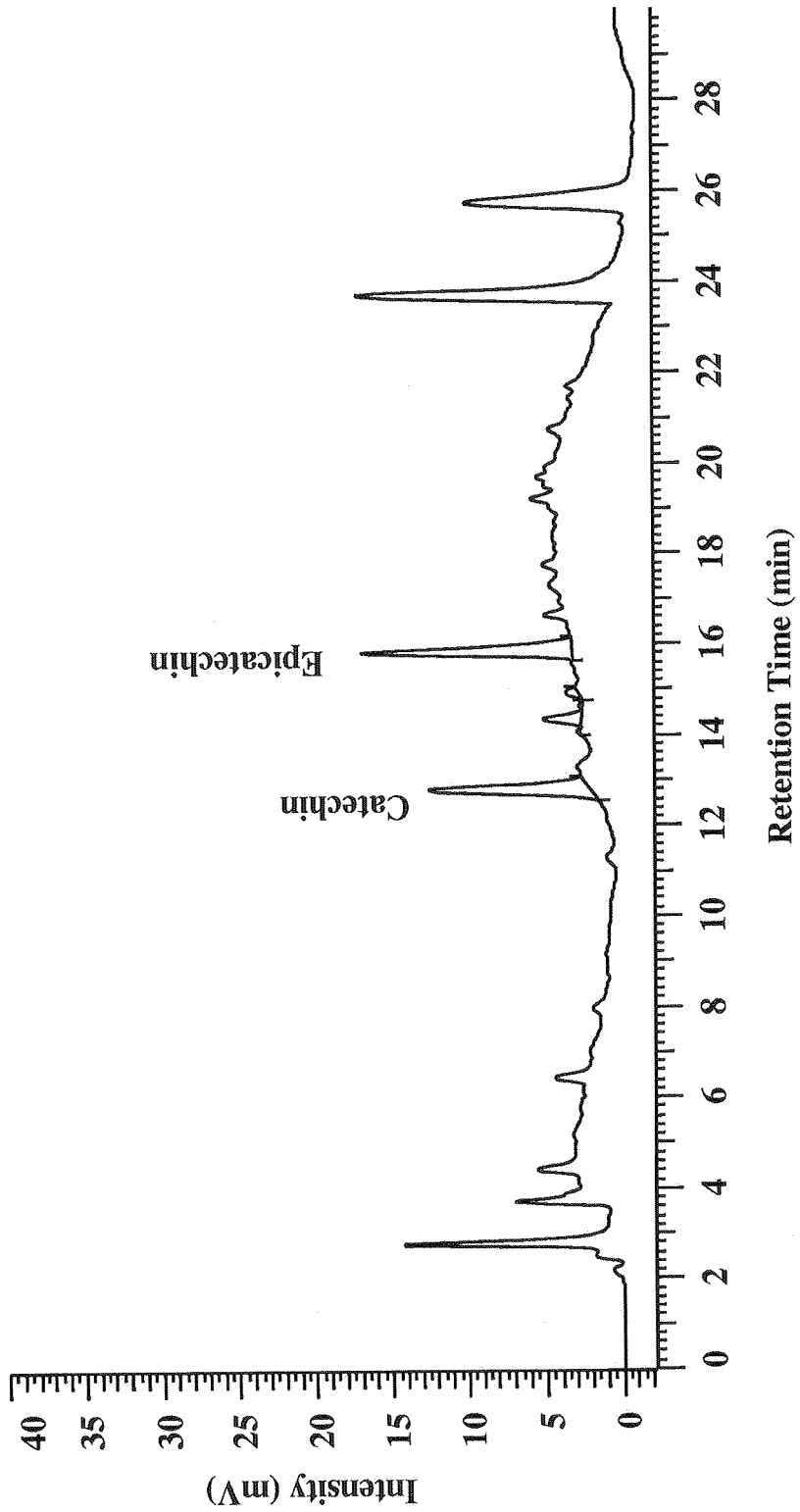

Intensity (mV)
40
35
30
25
20
15
10
5
0
Retention Time (min)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Catechin
Epicatechin

Figure 4B

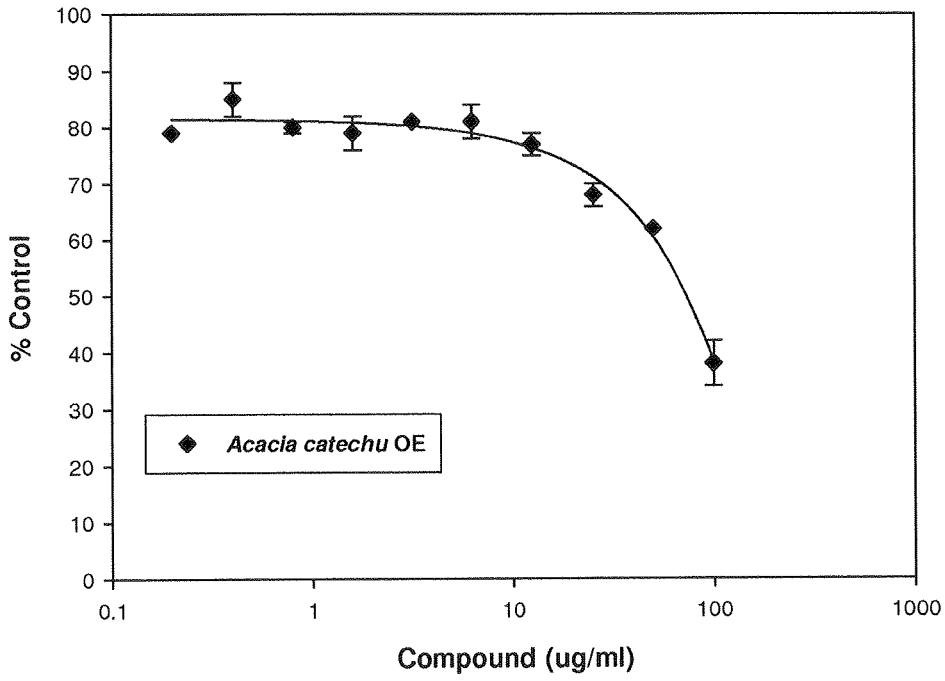

100
90
80
70
60
50
40
30
20
10
0
% Control
0.1
1
10
100
1000
Compound (ug/ml)
Acacia catechu OE

Figure 5

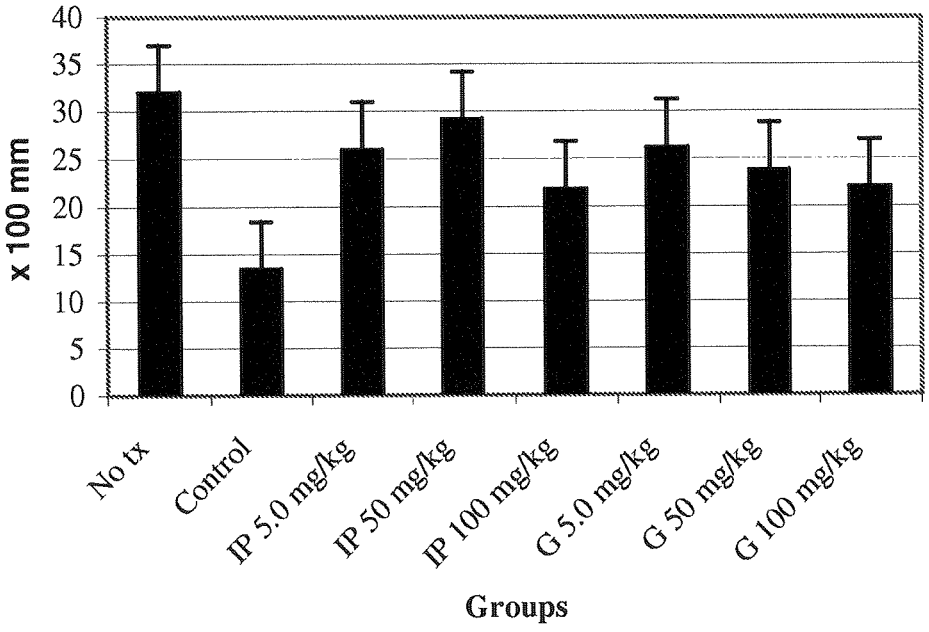

40
35
30
25
20
15
10
5
0
x 100 mm
No tx
Control
IP 5.0 mg/kg
IP 50 mg/kg
IP 100 mg/kg
G 5.0 mg/kg
G 50 mg/kg
G 100 mg/kg
Groups

Figure 6A

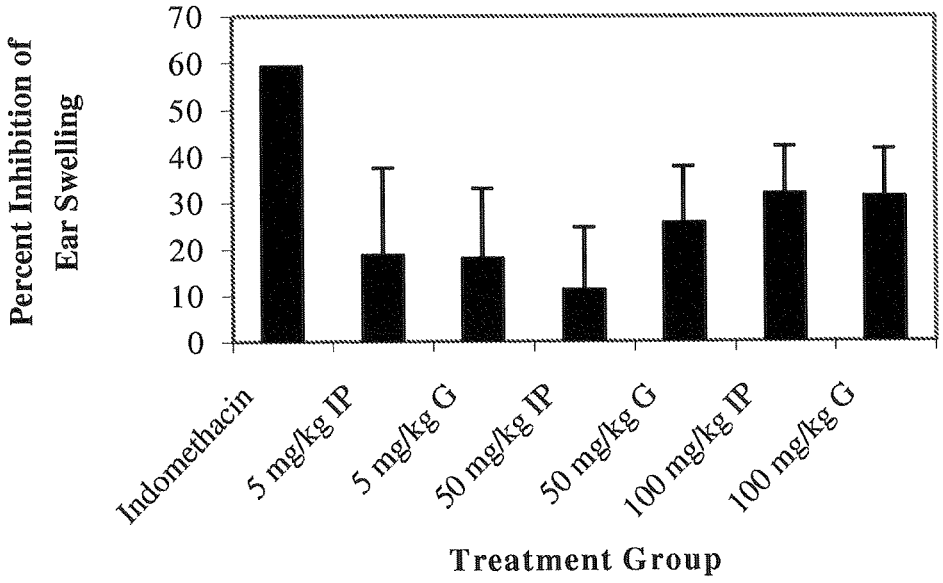

Percent Inhibition of Ear Swelling
70
60
50
40
30
20
10
0
Indomethacin
5 mg/kg IP
5 mg/kg G
50 mg/kg IP
50 mg/kg G
100 mg/kg IP
100 mg/kg G
Treatment Group

Figure 6B

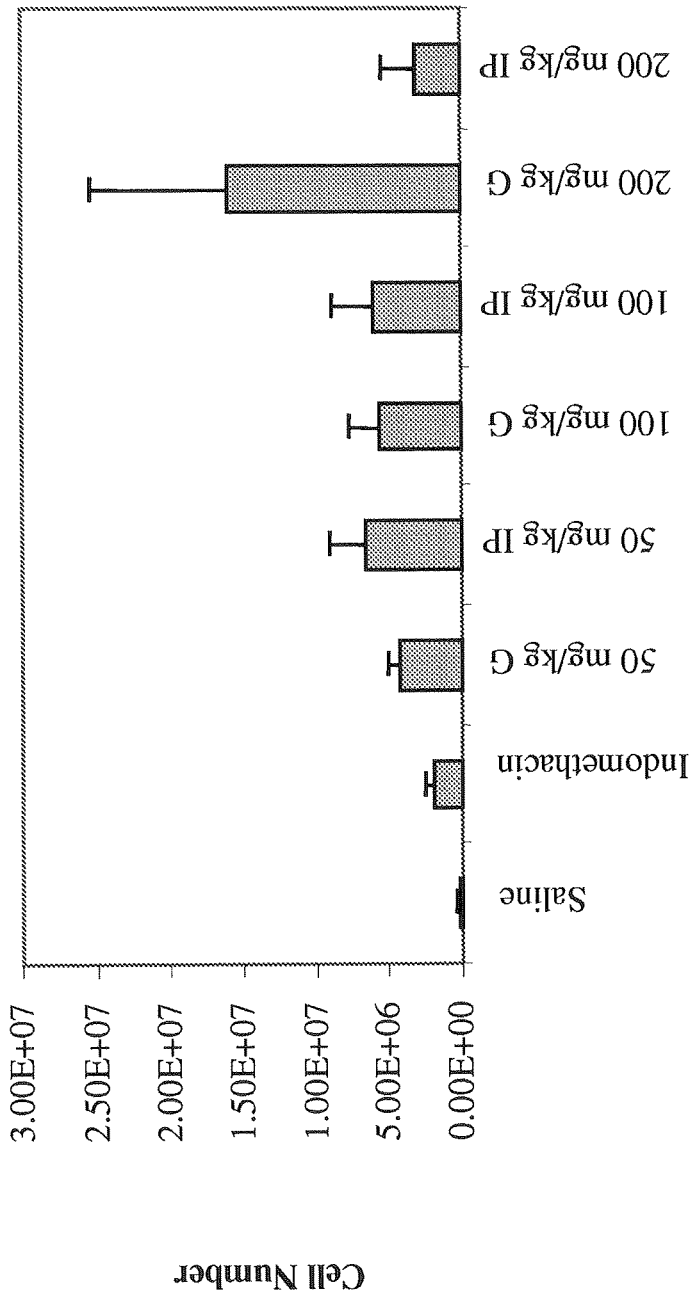

Cell Number
3.00E+07
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
Saline
Indomethacin
50 mg/kg G
50 mg/kg IP
100 mg/kg G
100 mg/kg IP
200 mg/kg G
200 mg/kg IP
Treatment Group

Figure 7A

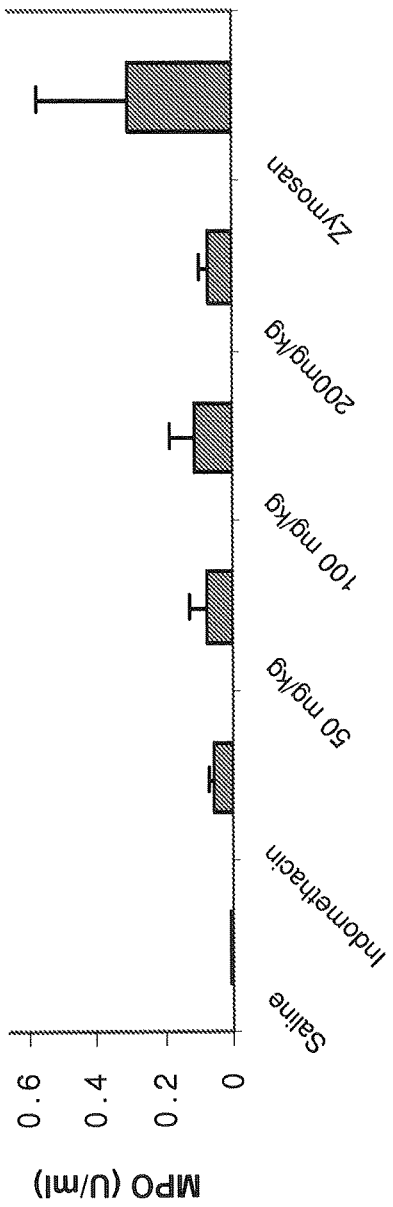

MPO (U/ml)
0.6
0.4
0.2
0
Saline
Indomethacin
50 mg/kg
100 mg/kg
200mg/kg
Zymosan

Figure 7B

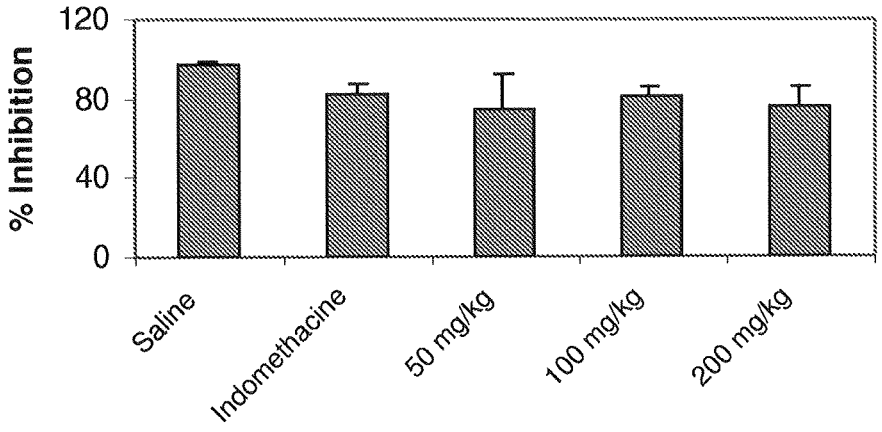

120
80
40
0
% Inhibition
Saline
Indomethacine
50 mg/kg
100 mg/kg
200 mg/kg

Figure 7C

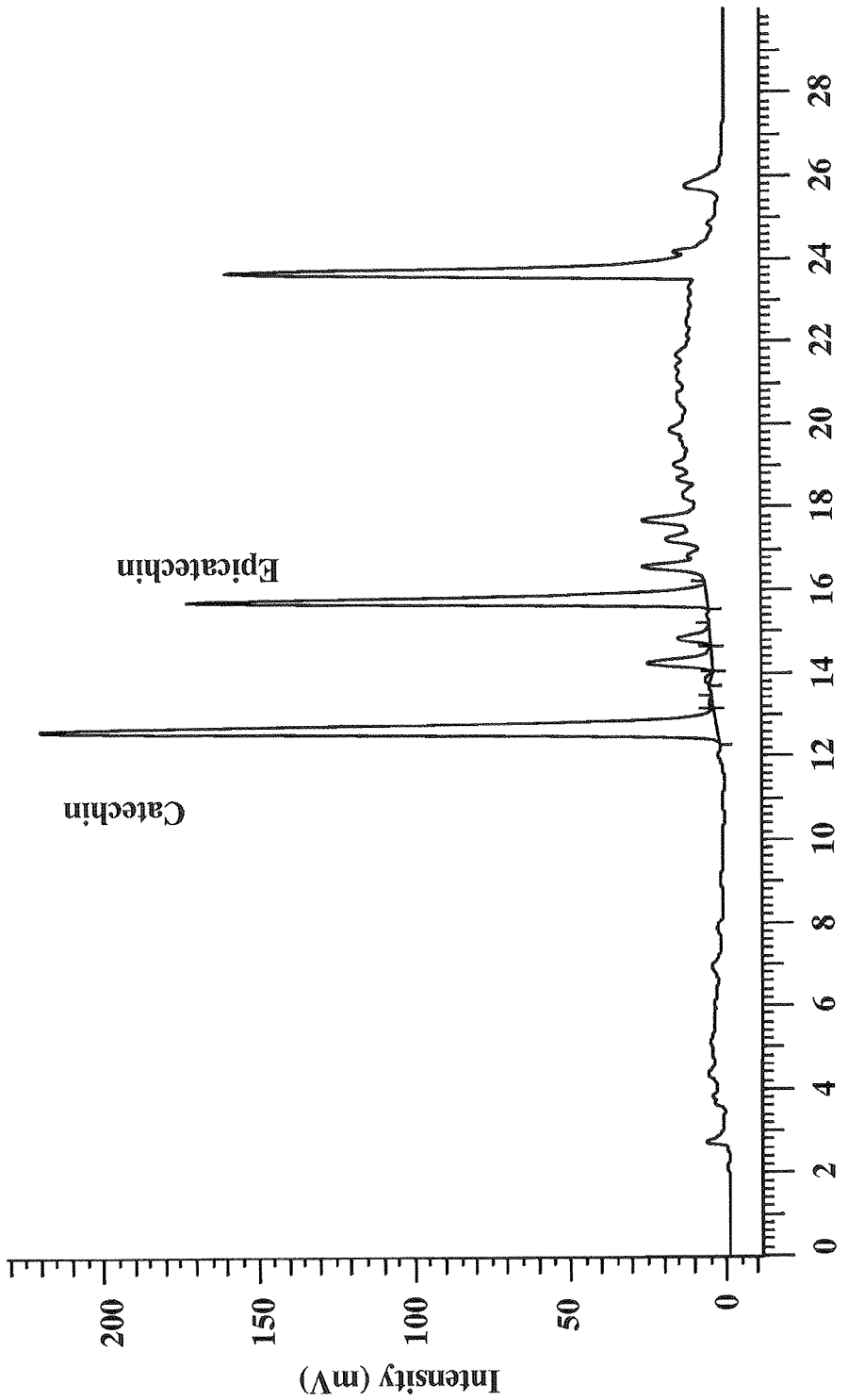

Catechin
Epicatechin
Intensity (mV)
200
150
100
50
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Retention Time (min)

Figure 8

ISOLATION OF A DUAL COX-2 AND 5-LIPDXYGENASE INHIBITOR FROM *ACACIA*

RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 13/355,362, filed Jan. 20, 2012 (now allowed); which is a continuation of U.S. patent application Ser. No. 11/457,388, filed Jul. 13, 2006 (now U.S. Pat. No. 8,124,134); which is a continuation of U.S. patent application Ser. No. 10/104,477, filed Mar. 22, 2002 (now U.S. Pat. No. 7,108,868). These applications are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

This invention relates generally to a method for the prevention and treatment diseases and conditions mediated by COX-2 and 5-lipoxygenase. Specifically, the present invention relates to a method for the prevention and treatment of COX-2 and 5-lipoxygenase mediated diseases and conditions by administration of a class of specific compounds, referred to as flavans, extracted from *Acacia* plants. Included in this invention is a method to generate standardized flavan extracts from plant sources.

BACKGROUND OF THE INVENTION

The liberation and metabolism of arachidonic acid (AA) from the cell membrane, results in the generation of pro-inflammatory metabolites by several different pathways. Arguably, two of the most important pathways to inflammation are mediated by the enzymes 5-lipoxygenase (5-LO) and cyclooxygenase (COX). These are parallel pathways that result in the generation of leukotrienes and prostaglandins, respectively, which play important roles in the initiation and progression of the inflammatory response. These vasoactive compounds are chemotaxins, which both promote infiltration of inflammatory cells into tissues and serve to prolong the inflammatory response. Consequently, the enzymes responsible for generating these mediators of inflammation have become the targets for many new drugs aimed at the treatment of inflammation, which that contributes to the pathogenesis of diseases such as rheumatoid arthritis, osteoarthritis, Alzheimer's disease and certain types of cancer.

Inhibition of the enzyme cyclooxygenase (COX) is the mechanism of action attributed to most nonsteroidal anti-inflammatory drugs (NSAIDS). There are two distinct isoforms of the COX enzyme (COX-1 and COX-2) that share approximately 60% sequence homology, but differ in expression profiles and function. COX-1 is a constitutive form of the enzyme that has been linked to the production of physiologically important prostaglandins, which help regulate normal physiological functions, such as platelet aggregation, protection of cell function in the stomach and maintenance of normal kidney function. (Dannhardt and Kiefer (2001) Eur. J. Med. Chem. 36:109-26). The second isoform, COX-2, is a form of the enzyme that is inducible by pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and other growth factors. (Herschmann (1994) Cancer Metastasis Rev. 134: 241-56; Xie et al. (1992) Drugs Dev. Res. 25:249-65). This isoform catalyzes the production of prostaglandin E2 (PGE2) from arachidonic acid (AA). Inhibition of COX-2 is responsible for the anti-inflammatory activities of conventional NSAIDs.

Inhibitors that demonstrate dual specificity for COX-2 and 5-LO while maintaining COX-2 selectivity relative to COX-1 would have the obvious benefit of inhibiting multiple pathways of arachidonic acid metabolism. Such inhibitors would block the inflammatory effects of PGE2, as well as, those of multiple leukotrienes (LT) by limiting their production. This includes the vasodilation, vasopermeability and chemotactic effects of LTB4 and LTD4 and the effects of LTE4, also known as the slow reacting substance of anaphalaxis. Of these, LTB4 has the most potent chemotactic and chemokinetic effects (Moore (1985) *Prostanoids: pharmacological, physiological and clinical relevance*. Cambridge University Press, N.Y., pp. 229-30) and has been shown to be elevated in the gastrointestinal mucosa of patients with inflammatory bowel disease (Sharon and Stenson (1983) Gastroenterology 84:1306-13) and within the synovial fluid of patients with rheumatoid arthritis. (Klicksein et al. (1980) J. Clin. Invest. 66:1166-70; Rae et al. (1982) Lancet ii:1122-4).

In addition to the above-mentioned benefits of dual COX-2/5-LO inhibitors, many dual inhibitors do not cause some of the side effects that are typical of NSAIDs or COX-2 inhibitors, including both the gastrointestinal damage and discomfort caused by traditional NSAIDs. It has been suggested that NSAID induced gastric inflammation is largely due to metabolites of 5-LO, particularly LTB4. (Kircher et al. (1997) Prostaglandins leukotrienes and essential fatty acids 56:417-23). Leukotrienes represent the primary arachidonic acid metabolites within the gastric mucosa following prostanoid inhibition. It appears that these compounds contribute to a significant amount of the gastric epithelial injury resulting from the use of NSAIDs. (Celotti and Laufer (2001) Pharmacological Research 43:429-36). Dual inhibitors of COX and 5-LO were also demonstrated to inhibit the coronary vasoconstriction in arthritic hearts in a rat model. (Gok et al. (2000) Pharmacology 60:41-46). Taken together, these characteristics suggest that there may be distinct advantages to dual inhibitors of COX-2 and 5-LO over COX-2 inhibitors and NSAIDs alone with regard to both increased efficacy and a lack of side effects.

Because the mechanism of action of COX inhibitors overlaps that of most conventional NSAID's, COX inhibitors are used to treat many of the same symptoms, including pain and swelling associated with inflammation in transient conditions and chronic diseases in which inflammation plays a critical role. Transient conditions include treatment of inflammation associated with minor abrasions, sunburn or contact dermatitis, as well as, the relief of pain associated with tension and migraine headaches and menstrual cramps. Applications to chronic conditions include arthritic diseases, such as rheumatoid arthritis and osteoarthritis. Although, rheumatoid arthritis is largely an autoimmune disease and osteoarthritis is caused by the degradation of cartilage in joints, reducing the inflammation associated with each provides a significant increase in the quality of life for those suffering from these diseases. (Wienberg (2001) Immunol. Res. 22:319-41; Wollhiem (2000) Curr. Opin. Rheum. 13:193-201). In addition to rheumatoid arthritis, inflammation is a component of rheumatic diseases in general. Therefore, the use of COX inhibitors has been expanded to include diseases, such as systemic lupus erythromatosus (SLE) (Goebel et al. (1999) Chem. Res. Tox. 12:488-500; Patrono et al. (1985) J. Clin. Invest. 76:1011-1018), as well as, rheumatic skin conditions, such as scleroderma. COX inhibitors are also used for the relief of inflammatory skin conditions that are not of rheumatic origin, such as psoriasis, in which reducing the inflammation resulting from the over production of prostaglandins could provide a direct benefit. (Fogh et al. (1993) Acta Derm

Venerologica 73:191-3). Simply stated COX inhibitors are useful for the treatment of symptoms of chronic inflammatory diseases, as well as, the occasional ache and pain resulting from transient inflammation.

In addition to their use as anti-inflammatory agents, another potential role for COX inhibitors is in the treatment of cancer. Over expression of COX-2 has been demonstrated in various human malignancies and inhibitors of COX-2 have been shown to be efficacious in the treatment of animals with skin, breast and bladder tumors. While the mechanism of action is not completely understood, the over expression of COX-2 has been shown to inhibit apoptosis and increase the invasiveness of tumorgenic cell types. (Dempke et al. (2001) J. Can. Res. Clin. Oncol. 127:411-17; Moore and Simmons (2000) Current Med. Chem. 7:1131-44). It is possible that enhanced production of prostaglandins resulting from the over expression of COX-2 promotes cellular proliferation and consequently, increases angiogenesis. (Moore (1985) in *Prostanoids: pharmacological, physiological and clinical relevance*, Cambridge University Press, N.Y., pp. 229-30; Fenton et al. (2001) Am. J. Clin. Oncol. 24:453-57).

There have been a number of clinical studies evaluating COX-2 inhibitors for potential use in the prevention and treatment of different types of cancer. In 1999, 130,000 new cases of colorectal cancer were diagnosed in the U.S. Aspirin, a non-specific NSAID, for example, has been found to reduce the incidence of colorectal cancer by 40-50% (Giovannucci et al. (1995) N Engl J. Med. 333:609-614) and mortality by 50% (Smalley et al. (1999) Arch Intern Med. 159:161-166). In 1999, the FDA approved the COX-2 inhibitor CeleCOXib for use in FAP (Familial Ademonatous Polyposis) to reduce colorectal cancer mortality. It is believed that other cancers, with evidence of COX-2 involvement, may be successfully prevented and/or treated with COX-2 inhibitors including, but not limited to esophageal cancer, head and neck cancer, breast cancer, bladder cancer, cervical cancer, prostate cancer, hepatocellular carcinoma and non-small cell lung cancer. (Jaeckel et al. (2001) Arch. Otolarnygol. 127:1253-59; Kirschenbaum et al. (2001) Urology 58:127-31; Dannhardt and Kiefer (2001) Eur. J. Med. Chem. 36:109-26). COX-2 inhibitors may also prove successful in preventing colon cancer in high-risk patients. There is also evidence that COX-2 inhibitors can prevent or even reverse several types of life-threatening cancers. To date, as many as fifty studies show that COX-2 inhibitors can prevent premalignant and malignant tumors in animals, and possibly prevent bladder, esophageal and skin cancers as well. COX-2 inhibition could prove to be one of the most important preventive medical accomplishments of the century.

Recent scientific progress has identified correlations between COX-2 expression, general inflammation and the pathogenesis of Alzheimer's disease (AD). (Ho et al. (2001) Arch. Neurol. 58:487-92). In animal models, transgenic mice that over express the COX-2 enzyme have neurons that are more susceptible to damage. The National Institute on Aging (NIA) is launching a clinical trial to determine whether NSAIDs can slow the progression of Alzheimer's disease. Naproxen (a non-selective NSAID) and rofecoxib (Vioxx, a COX-2 specific selective NSAID) will be evaluated. Previous evidence has indicated inflammation contributes to Alzheimer's disease. According to the Alzheimer's Association and the NIA, about 4 million people suffer from AD in the U.S.; and this is expected to increase to 14 million by mid-century.

The COX enzyme (also known as prostaglandin H2 synthase) catalyzes two separate reactions. In the first reaction, arachidonic acid is metabolized to form the unstable prostaglandin G2 (PGG2), a cyclooxygenase reaction. In the second reaction, PGG2 is converted to the endoperoxide PGH2, a peroxidase reaction. The short-lived PGH2 non-enzymatically degrades to PGE2. The compounds described herein are the result of a discovery strategy that combined an assay focused on the inhibition of COX-1 and COX-2 peroxidase activity with a chemical dereplication process to identify novel inhibitors of the COX enzymes.

Acacia is a genus of leguminous trees and shrubs. The genus *Acacia* includes more than 1000 species belonging to the family of Leguminosae and the subfamily of Mimosoideae. Acacias are distributed worldwide in tropical and subtropical areas of central and south America, Africa, parts of Asia, as well as, Australia, which has the largest number of endemic species. Acacias occur primarily in dry and arid regions, where the forests are often in the nature of open thorny shrubs. The genus *Acacia* is divided into 3 subgenera based mainly on the leaf morphology—*Acacia, Aculiferum* and *Heterophyllum*. Based on the nature of the leaves of mature trees, however, the genus *Acacia* can be divided into two "popular" groups: the typical bipinnate leaved species and the phyllodenous species. A phyllode is a modified petiole expanded into a leaflike structure with no leaflets, an adaptation to xerophytic conditions. The typical bipinnate leaved species are found primarily throughout the tropics, whereas the phyllodenous species occur mainly in Australia. More than 40 species of *Acacia* have been reported in India. Gamble in his *Flora of Madras Presidency* listed 23 native species for southern India, 15 of which are found in Tamil Nadu. Since that time, many new *Acacia* species have been introduced to India. Approximately 40 species are now found in Tamil Nadu itself. The indigenous species are primarily thorny trees or shrubs and a few are thorny stragglers, such as *A. caesia, A. pennata* and *A. sinuata*. Many species have been introduced from Africa and Australia, i.e. *Acacia mearnsii, A. picnantha* and *A. dealbata*, which have bipinnate leaves and *A. auriculiformis, A. holoserecia* and *A. mangium*, which are phyllodenous species.

Acacias are very important economically, providing a source of tannins, gums, timber, fuel and fodder. Tannins, which are isolated primarily from bark, are used extensively for tanning hides and skins. Some *Acacia* barks are also used for flavoring local spirits. Some indigenous species like *A. sinuata* also yield saponins, which are any of various plant glucosides that form soapy lathers when mixed and agitated with water. Saponins are used in detergents, foaming agents and emulsifiers. The flowers of some *Acacia* species are fragrant and used to make perfume. For example, Cassie perfume is obtained from *Acacia ferrugenea*. The heartwood of many Acacias is used for making agricultural implements and also provides a source of firewood. *Acacia* gums find extensive use in medicine and confectionary and as sizing and finishing materials in the textile industry. Lac insects can be grown on several species, including *A. nilotica* and *A. catechu*. Some species have been used for forestation of wastelands, including *A. nilotica*, which can withstand some water inundation and a few such areas have become bird sanctuaries.

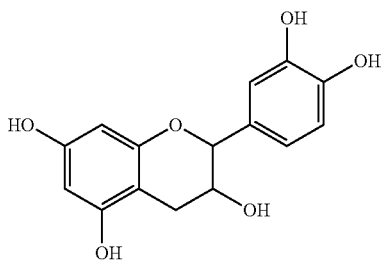
To date, approximately 330 compounds have been isolated from various *Acacia* species. Flavonoids, a type of water-soluble plant pigments, are the major class of compounds isolated from Acacias. Approximately 180 different flavonoids have been identified, 111 of which are flavans. Terpenoids are second largest class of compounds isolated from species of the *Acacia* genus, with 48 compounds having been identified. Other classes of compounds isolated from *Acacia* include, alkaloids (28), amino acids/peptides (20), tannins (16), carbohydrates (15), oxygen heterocycles (15) and aliphatic compounds (10). (Buckingham, *The Combined Chemical Dictionary*, Chapman & Hall CRC, version 5:2, December 2001).

Phenolic compounds, particularly flavans are found in moderate to high concentrations in all *Acacia* species. (Abdulrazak et al. (2000) Journal of Animal Sciences. 13:935-940). Historically, most of the plants and extracts of the *Acacia* genus have been utilized as astringents to treat gastrointestinal disorders, diarrhea and indigestion and to stop bleeding. (Vautrin (1996) Universite Bourgogne (France) European abstract 58-01C:177; Saleem et al. (1998) Hamdard Midicus. 41:63-67). The bark and pods of *Acacia arabica* Willd contain large quantities of tannins and have been utilized as astringents and expectorants. (Nadkarni (1996) India Materia Medica, Bombay Popular Prakashan, pp. 9-17). Diarylpropanol derivatives, isolated from stem bark of *Acacia tortilis* from Somalia, have been reported to have smooth muscle relaxing effects. (Hagos et al. (1987) Planta Medica. 53:27-31, 1987). It has also been reported that terpenoid saponins isolated from *Acacia victoriae* have an inhibitory effect on dimethylbenz(a)anthracene-induced murine skin carcinogenesis (Hanausek et al. (2000) Proceedings American Association for Cancer Research Annual Meeting 41:663) and induce apotosis (Haridas et al. (2000) Proceedings American Association for Cancer Research Annual Meeting. 41:600). Plant extracts from *Acacia nilotica* have been reported to have spasmogenic, vasoconstrictor and antihypertensive activity (Amos et al. (1999) Phytotherapy Research 13:683-685; Gilani et al. (1999) Phytotherapy Research. 13:665-669), and antiplatelet aggregatory activity (Shah et al. (1997) General Pharmacology. 29:251-255). Anti-inflammatory activity has been reported for *A. nilotica*. It was speculated that flavonoids, polysaccharides and organic acids were potential active components. (Dafallah and Al-Mustafa (1996) American Journal of Chinese Medicine. 24:263-269). To date, the only reported 5-lipoxygenase inhibitor isolated from *Acacia* is a monoterpenoidal carboxamide (Seikine et al. (1997) Chemical and Pharmaceutical Bulletin. 45:148-11).

Acacia gums have been formulated with other plant ingredients and used for ulcer prevention without identification of any of the active components. (Fuisz, U.S. Pat. No. 5,651,987). *Acacia* gums have also been formulated with other plant ingredients and used to improve drug dissolution (Blank, U.S. Pat. No. 4,946,684), by lowering the viscosity of nutritional compositions (Chancellor, U.S. Pat. No. 5,545,411).

The extract from the bark of *Acacia* has been patented in Japan for external use as a whitening agent (Abe, JP10025238), as a glucosyl transferase inhibitor for dental applications (Abe, JP07242555), as a protein synthesis inhibitor (Fukai, JP 07165598), as an active oxygen scavenging agent for external skin preparations (Honda, JP 07017847, Bindra U.S. Pat. No. 6,1266,950), and as a hyaluronidase inhibitor for oral consumption to prevent inflammation, pollinosis and cough (Ogura, JP 07010768).

Catechin is a flavan, found primarily in green tea, having the following structure.



Catechin

Catechin works both alone and in conjunction with other flavonoids found in tea, and has both antiviral and antioxidant activity. Catechin has been shown to be effective in the treatment of viral hepatitis. It also appears to prevent oxidative damage to the heart, kidney, lungs and spleen. Catechin also has been shown to inhibit the growth of stomach cancer cells.

Catechin and its isomer epicatechin inhibit prostaglandin endoperoxide synthase with an IC_{50} value of 40 μmol/L. (Kalkbrenner et al. (1992) Pharmacol. 44:1-12). Five flavan-3-ol derivatives, including (+)-catechin and gallocatechin, isolated from four plant species: *Atuna racemosa, Syzygium carynocarpum, Syzygium malaccense* and *Vantanea peruviana*, exhibit equal to weaker inhibitory activity against COX-2, relative to COX-1, with IC_{50} values ranging from 3.3 μM to 138 μM (Noreen et al. (1998) Planta Med. 64:520-524). (+)-Catechin, isolated from the bark of *Ceiba pentandra*, inhibits COX-1 with an IC_{50} value of 80 μM (Noreen et al. (1998) J. Nat. Prod. 61:8-12). Commercially available pure (+)-catechin inhibits COX-1 with an IC_{50} value of around 183 to 279 μM depending upon the experimental conditions, with no selectivity for COX-2. (Noreen et al. (1998) J. Nat. Prod. 61:1-7).

Green tea catechin, when supplemented into the diets of Dawley male rats, lowered the activity level of platelet phospholipase A2 and significantly reduced platelet cyclooxygenase levels. (Yang et al. (1999) J. Nutr. Sci. Vitaminol. 45:337-346). Catechin and epicatechin reportedly weakly suppress COX-2 gene transcription in human colon cancer DLD-1 cells (IC_{50}=415.3 μM). (Mutoh et al. (2000) Jpn. J. Cancer Res. 91:686-691). The neuroprotective ability of (+)-catechin from red wine results from the antioxidant properties of catechin, rather than inhibitory effects on intracellular enzymes, such as cyclooxygenase, lipoxygenase, or mitric oxide synthase (Bastianetto et al. (2000) Br. J. Pharmacol. 131:711-720). Catechin derivatives purified from green tea and black tea, such as epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and theaflavins showed inhibition of cyclooxygenase and lipoxygenase dependent metabolism of arachidonic acid in human colon mucosa and colon tumor tissues (Hong et al. (2001) Biochem. Pharmacol. 62:1175-1183) and induce COX-2 expression and PGE(2) production (Park et al. (2001) Biochem. Biophys. Res. Commun. 286:721-725). Epiafzelechin isolated from the aerial parts of *Celastrus orbiculatus* exhibited a dose-dependent inhibition of COX-1 activity with an IC_{50} value of 15 μM and also demonstrated anti-inflammatory activity against carrageenin-induced mouse paw edema following oral administration at a dosage of 100 mg/kg. (Min et al. (1999) Planta Med. 65:460-462).

Catechin and its derivatives from various plant sources, especially from green tea leaves, have been used in the treatment of HPV infected Condyloma acuminata (Cheng, U.S. Pat. No. 5,795,911) and in the treatment of hyperplasia caused by papilloma virus (Cheng, U.S. Pat. Nos. 5,968,973 and 6,197,808). Catechin and its derivatives have also been used topically to inhibit angiogenesis in mammalian tissue, such as skin cancer, psoriasis, spider veins or under eye circles (Anderson, U.S. Pat. No. 6,248,341), against UVB-induced tumorigenesis on mice (Agarwal et al. (1993) Photochem. Photobiol. 58:695-700), for inhibiting nitric oxide synthase at the level of gene expression and enzyme activity (Chan, U.S. Pat. No. 5,922,756), as a hair-growing agent (Takahashi, U.S. Pat. No. 6,126,940). Catechin based compositions have also been formulated with other extracts and vitamins for treatment of acne (Murad U.S. Pat. No. 5,962,517), hardening the tissue of digestive organs (Shi, U.S. Pat. No. 5,470,589), for inhibiting 5 alpha-reductase activity in

treating androgenic disorder related diseases and cancers (Liao, U.S. Pat. No. 5,605,929). Green tea extract has been formulated with seven other plant extracts for reducing inflammation by inhibiting the COX-2 enzyme, without identification of any of the specific active components (Mewmark, U.S. Pat. No. 6,264,995).

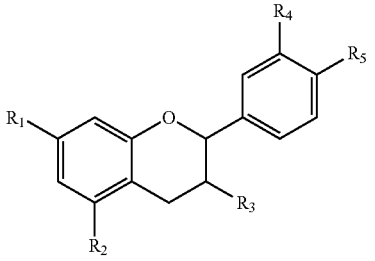
To date, a number of naturally occurring flavonoids have been commercialized for varying uses. For example, liposome formulations of *Scutellaria* extracts have been utilized for skin care (U.S. Pat. Nos. 5,643,598; 5,443,983). Baicalin has been used for preventing cancer, due to its inhibitory effects on oncogenes (U.S. Pat. No. 6,290,995). Baicalin and other compounds have been used as antiviral, antibacterial and immunomodulating agents (U.S. Pat. No. 6,083,921) and as natural anti-oxidants (Poland Pub. No. 9,849,256). Chrysin has been used for its anxiety reducing properties (U.S. Pat. No. 5,756,538). Anti-inflammatory flavonoids are used for the control and treatment of anorectal and colonic diseases (U.S. Pat. No. 5,858,371), and inhibition of lipoxygenase (U.S. Pat. No. 6,217,875). These compounds are also formulated with glucosamine collagen and other ingredients for repair and maintenance of connective tissue (Bath, U.S. Pat. No. 6,333,304). Flavonoid esters constitute active ingredients for cosmetic compositions (U.S. Pat. No. 6,235,294). The bark, extract and compounds derived from *Phellodendron amurense* have been patented for use in treatment of inflammatory diseases (U.S. Pat. Nos. 5,766,614; 5,908,628; 6,113,909; 6,193,977). Chemy bioflavonoids from *Prunus avium* and *Prunus cerasus* with anthocyanidin type of structures have been patented as cyclooxygenase inhibitors (U.S. Pat. No. 6,194,469, U.S. Pat. Appl. 20010002407).

SUMMARY OF THE INVENTION

The present invention includes methods that are effective in simultaneously inhibiting the enzymes COX-2 and 5-lipoxygenase. The method for simultaneous dual inhibition of the enzymes COX-2 and 5-LO is comprised of administering a composition comprising an individual and/or a mixture of multiple flavans isolated from a single plant or multiple plants in the *Acacia* genus of plants to a host in need thereof.

The present also includes methods for the prevention and treatment of COX-2 and 5-LO mediated diseases and conditions. The method for preventing and treating COX-2 and 5-LO mediated diseases and conditions is comprised of administering to a host in need thereof an effective amount of a composition comprising an individual and/or a mixture of multiple flavans isolated from a single plant or multiple plants in the *Acacia* genus of plants and a pharmaceutically acceptable carrier.

The flavans that can be used in accordance with the following include compounds illustrated by the following general structure:



wherein

R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of H, —OH, —SH, —OCH_3, —SCH_3, —OR, —SR, —NH_2, —NRH, —NR_2, —$NR_3^+X^-$, esters of the mentioned substitution groups, including, but not limited to, gallate, acetate, cinnamoyl and hydroxyl-cinnamoyl esters, trihydroxybenzoyl esters and caffeoyl esters; thereof carbon, oxygen, nitrogen or sulfur glycoside of a single or a combination of multiple sugars including, but not limited to, aldopentoses, methyl aldopentose, aldohexoses, ketohexose and their chemical derivatives thereof; dimer, trimer and other polymerized flavans;

wherein

R is an alkyl group having between 1-10 carbon atoms; and

X is selected from the group of pharmaceutically acceptable counter anions including, but not limited to hydroxyl, chloride, iodide, sulfate, phosphate, acetate, fluoride, carbonate, etc.

The method of this invention can be used to treat and prevent a number of COX-2 and 5-LO mediated diseases and conditions including, but not limited to, osteoarthritis, rheumatoid arthritis, menstrual cramps, systemic lupus erythromatosus, psoriasis, chronic tension headaches, migraine headaches, topical wound and minor inflammatory conditions, inflammatory bowel disease and solid cancers.

As noted above the flavans of this invention may be obtained from a plant or plants selected from the genus of *Acacia*. In a preferred embodiment, the plant is selected from the group consisting of *Acacia catechu, Acacia concinna, Acacia farnesiana, Acacia Senegal, Acacia speciosa, Acacia arabica, A. caesia, A. pennata, A. sinuata. A. mearnsii, A. picnantha, A. dealbata, A. auriculiformis, A. holoserecia* and *A. mangium.*

The compositions of this invention can be administered by any method known to one of ordinary skill in the art. The modes of administration include, but are not limited to, enteral (oral) administration, parenteral (intravenous, subcutaneous, and intramuscular) administration and topical application. The method of treatment according to this invention comprises administering internally or topically to a patient in need thereof a therapeutically effective amount of the individual and/or a mixture of multiple flavans from a single source or multiple sources that include, but not limited to, synthetically obtained, naturally occurring, or any combination thereof.

The present invention includes a method for isolating and purifying flavans from the *Acacia* genus of plants. The method of the present invention comprises: a) extracting the ground biomass of a plant selected from the *Acacia* genus of plants; b) neutralizing and concentrating said extract; and c) purifying said neutralized and concentrated extract. In a preferred embodiment of the invention the extract is purified using a method selected from the group consisting of recrystallization, precipitation, solvent partition and/or chromatographic separation. The present invention provides a commercially viable process for the isolation and purification of *Acacia* flavans having desirable physiological activity.

The present invention implements a strategy that combines a series of biomolecular screens with a chemical dereplication process to identify active plant extracts and the particular compounds within those extracts that specifically inhibit COX-2 and 5-LO enzymatic activity and inflammation. A total of 1230 plant extracts were screened for their ability to inhibit the peroxidase activity associated with recombinant COX-2. This primary screen identified 22 plant extracts that were further studied for their ability to specifically and selectively inhibit COX-2 in vitro in both cell based and whole blood assays. Those extracts that were efficacious in vitro

were then tested for their ability to inhibit inflammation in vivo using a both air pouch and topical ear-swelling models of inflammation when administered by multiple routes (IP and oral). These studies resulted in the discovery of botanical extracts that inhibited COX-2 activity and were efficacious both in vitro and in vivo. These extracts were then tested for their ability to inhibit 5-LO. These studies resulted in the identification of flavan extracts from the *Acacia* genus of plants that demonstrate dual specificity for COX-2 and 5-LO. Applicant believes that this is first report of a composition of matter isolated from the *Acacia* genus of plants that demonstrates this dual specificity for COX-2 and 5-LO.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** depicts graphically a profile of the inhibition of COX-1 and COX-2 by HTP fractions from organic extracts of *Acacia catechu*. The extracts were examined for their inhibition of the peroxidase activity of recombinant ovine COX-1 (■) or ovine COX-2 (●). The data is presented as percent of untreated control.

FIG. **2** depicts graphically a profile of the inhibition of COX-1 and COX-2 by HTP fractions from aqueous extracts of *Acacia catechu*. The extracts were examined for their inhibition of the peroxidase activity of recombinant ovine COX-1 (■) or ovine COX-2 (●). The data is presented as percent of untreated control.

FIG. **3** depicts the results of LC/PDA/MS analysis of active HTP fraction D11, which is described in Example 3. FIG. **3**A depicts the LC/PDA chromatogram of HTP fraction D11. FIG. **3**B depicts the selected ion chromatogram of HTP fraction D11 at m/z=579 and the structure of the compound robinetinidol-(4-6/8)-catechin. FIG. **3**C depicts the selected ion chromatogram of HTP fraction D11 at m/z=291 and the structure of the compound catechin.

FIG. **4** depicts the high-pressure liquid chromatography (HPLC) traces of the organic (FIG. **4**A) and aqueous (FIG. **4**B) extracts from *Acacia catechu*.

FIG. **5** illustrates graphically the effect of *Acacia catechu* organic extracts on 5-lipoxygenase activity.

FIG. **6** illustrates the inhibition of arachidonic acid induced inflammation by *Acacia catechu*. The in vivo efficacy was evaluated based on the ability to inhibit swelling induced by direct application of arachidonic acid as described in Example 9. The average difference in swelling between treated ears and control ears is depicted in FIG. **6**A. FIG. **6**B depicts the percent inhibition of each group in comparison to the arachidonic acid treated control.

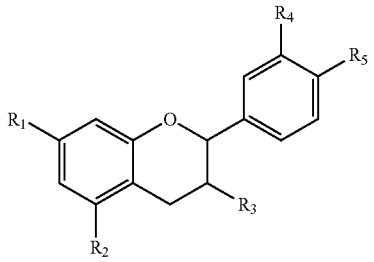
FIG. **7** illustrates the effect of standardized *Acacia* extracts on Zymosan induced inflammation. Zymosan was used to elicit a pro-inflammatory response in an air pouch as described in Example 9. Markers of inflammation including infiltration of pro-inflammatory cells (FIG. **7**A), MPO concentrations under different experimental conditions (FIG. **7**B) and percent inhibition of MPO activity within the air pouch fluid (FIG. **7**C) were used to evaluate the efficacy and mechanism of action of the anti-inflammatory activity of *Acacia* extracts.

FIG. **8** depicts the HPLC tract of the flavans extracted from *Acacia catechu* with 80% MeOH in water.

DETAILED DESCRIPTION OF THE INVENTION

Various terms are used herein to refer to aspects of the present invention. To aid in the clarification of the description of the components of this invention, the following definitions are provided.

"Flavans" are a specific class of flavonoids, which can be generally represented by the following general structure:



wherein

R_1, R_2, R_3, R_4 and R_5 are independently selected from the group consisting of H, —OH, —SH, —OCH_3, —SCH_3, —OR, —SR, —NH_2, —NRH, —NR_2, —$NR_3^+X^-$, esters of substitution groups, including, but not limited to, gallate, acetate, cinnamoyl and hydroxyl-cinnamoyl esters, trihydroxybenzoyl esters and caffeoyl esters; thereof carbon, oxygen, nitrogen or sulfur glycoside of a single or a combination of multiple sugars including, but not limited to, aldopentoses, methyl aldopentose, aldohexoses, ketohexose and their chemical derivatives thereof; dimer, trimer and other polymerized flavans;

wherein

R is an alkyl group having between 1-10 carbon atoms; and

X is selected from the group of pharmaceutically acceptable counter anions including, but not limited to hydroxyl, chloride, iodide, sulfate, phosphate, acetate, fluoride, carbonate, etc.

"Therapeutic" as used herein, includes treatment and/or prophylaxis. When used, therapeutic refers to humans, as well as, other animals.

"Pharmaceutically or therapeutically effective dose or amount" refers to a dosage level sufficient to induce a desired biological result. That result may be the delivery of a pharmaceutical agent, alleviation of the signs, symptoms or causes of a disease or any other desired alteration of a biological system.

A "host" is a living subject, human or animal, into which the compositions described herein are administered.

Note, that throughout this application various citations are provided. Each citation is specifically incorporated herein in its entirety by reference.

The present invention includes methods that are effective in simultaneously inhibiting both the cyclooxygenase (COX-2) and 5-lipoxygenase (5-LO) enzymes. The method for the simultaneous dual inhibition of the COX-2 and 5-LO enzymes is comprised of administering a composition comprising an individual and/or a mixture of multiple flavans isolated from a single plant or multiple plants selected from the *Acacia* genus of plants to a host in need thereof.

The present also includes methods for the prevention and treatment of COX-2 and 5-LO mediated diseases and conditions. The method for preventing and treating COX-2 and 5-LO mediated diseases and conditions is comprised of administering to a host in need thereof an effective amount of

a composition comprising an individual and/or a mixture of multiple flavans isolated from a single plant or multiple plants selected from the *Acacia* genus of plants and a pharmaceutically acceptable carrier.

The flavans that can be used in accordance with the method of this invention include compounds illustrated by the general structure set forth above. The flavans of this invention are isolated from a plant or plants selected from the *Acacia* genus of plants. In a preferred embodiment, the plant is selected from the group consisting of *Acacia catechu, Acacia concinna, Acacia farnesiana, Acacia Senegal, Acacia speciosa, Acacia arabica, A. caesia, A. pennata, A. sinuata. A. mearnsii, A. picnantha, A. dealbata, A. auriculiformis, A. holosericia* and *A. mangium.*

The flavans can be found in different parts of plants, including but not limited to stems, stem barks, trunks, trunk barks, twigs, tubers, roots, root barks, young shoots, seeds, rhizomes, flowers and other reproductive organs, leaves and other aerial parts.

In order to identify compounds able to inhibit the COX enzymes an extract library composed of 1230 extracts from 615 medicinal plants collected from China, India, and other countries was created. This primary screen identified 22 plant extracts that were further studied for their ability to specifically and selectively inhibit COX-2 in vitro in both cell based and whole blood assays. A general method for preparing the extracts is described in Example 1, which uses the *Acacia catechu* species for purposes of illustration. The ground powder of the bark from *Acacia catechu* was extracted with an organic solvent followed by deionized (DI) water. The extraction process yields an organic and an aqueous extract for each species examined. The results of the extraction are set forth in Table 1. These primary extracts are the source material used in the preliminary assay to identify inhibitors of the cyclooxygenase enzyme's peroxidase activity, which is one of the main functional activities of cyclooxygenase and is responsible for converting PGG2 to PGH2 and ultimately PGE2, as described in detail above. This assay is described in Example 2 and the results are set forth in Table 2. With reference to Table 2, it can be seen that the organic and aqueous extracts from *Acacia catechu* inhibited the peroxidase activity of COX-2 albeit to differing degrees.

The COX-2 inhibitory activity from the primary assay of the crude extracts was confirmed by measurement of dose response and IC_{50} (the concentration required to inhibit 50% of the enzyme's activity). The IC_{50} values are set forth in Table 3. In this assay, *Acacia catechu* organic extract was efficacious (IC_{50}=3/6 μg/mL) against human/ovine COX-2 and ovine COX-1 (IC_{50}=2.5 μg/mL). Thus, the primary screens for inhibitors of the COX enzyme revealed that the organic extract from *Acacia* genus was efficacious against COX-2 enzyme.

In order to efficiently identify active compounds from plant extracts, a high throughput fractionation process was used, as described in Example 3. Briefly, the organic and aqueous extracts from *Acacia catechu* were fractionated with a high throughput purification system coupled with a due channel broadband UV detector and two Gilson 222XL liquid handlers. Eighty-eight fractions were collected in two 96 deep well plates. Each of the fractions was tested for its ability to inhibit COX activity as per the primary assay, as described in Example 4 and structure dereplication was initialized for the positive HTP fractions with off-line LC/PDA and LC/MS analyses. The results are set forth in FIGS. **1** and **2**, which depict the profile of COX-1 and COX-2 inhibition by various HTP fractions derived from the organic and aqueous extracts of *Acacia catechu*, respectively. It should be noted that a number of the HTP fractions actually exhibit selective COX-2 inhibitory activity, suggesting that there are multiple compounds in the extracts that contribute to the observed selective inhibitory effects of the whole extract.

The active HTP fractions from the aqueous extracts labeled well # C8 to F7 were analyzed using LC/MS with positive mode. The LC/PDA/PDA/MS analysis of fraction D11 are set forth in FIG. **3**. The first active peak was located between fractions C9 to D9 and contained catechin components with a $[molecular\ ion+1]^+$ at 291 m/z. The second active peak, which exhibited COX-1 selectivity, was located between fractions D9 to E2 and contained catechin dimer, having a molecular ion at 578 m/z. The third active peak, which exhibited COX-2 selectivity, was located between fractions #E8 to F8 and contained multiple components with an m/z at 573 & 579, which corresponds to 4-hydroxycinnamoyl-oleanen-3-ol and robinetinidol-catechin, respectively.

The separation, purification and identification of the active components present in the organic extract of *Acacia catechu* is described in Example 5. Using the methodology described in Example 5, catechin and epicatechin were identified as the two major active compounds in the organic extract from the roots of *Acacia catechu*, having IC_{50} values of 5-7 μg/mL.

HPLC quantification of the active extracts from *Acacia catechu* is described in Example 6. The results are set forth in Table 4 which shows that the flavan content in the organic and aqueous extracts, as determined by HPLC, is 30.4% and 1.0%, respectively. This explains why the inhibitory activity of the organic extract is more than twice that of the aqueous extract. HPLC analysis also demonstrates that each extract contains minor flavan components, which contribute to the selective COX-2 inhibitory activity. The HPLC results are set forth in FIGS. **4**A and B.

The primary assay described in Example 2 is a cell free system utilizing recombinant enzymes. To further demonstrate the biological activity of the active extracts and compounds, two models that represent cell based in vitro efficacy and animal based in vivo efficacy were employed. The method used to evaluate in vitro efficacy and selectivity is described in Example 7. Two cell lines were selected that could be induced to express primarily COX-1 (THP-1 cells) and COX-2 (HOSC cells), respectively. Each cell type was examined for the production of PGE_2, the primary product of the COX enzymes. The results are set forth in Table 5, which shows that the *Acacia* organic extract inhibits both the COX-1 and COX-2 enzymes with a preference for the COX-1 enzyme. While the use of the THP-1 cell line is important and demonstrates the ability of the active compounds to cross the cell membrane, it is an immortalized cell line therefore evaluation of efficacy based on a more relevant model system is desirable. As a result, the extract was also evaluated using whole blood as the primary source of both COX-1 and COX-2 activity. This system measures the inhibition of the production of PGE_2 vs. TXB_2 to differentiate between COX-2 and COX-1 inhibitory activity, respectively. The results, which are set forth in Table 5, demonstrate that both the COX-1 and COX-2 enzymes are inhibited by the *Acacia* extracts. The IC_{50} values suggest that within this system the *Acacia* extracts are more efficacious against COX-2. Taken as a whole, the inhibitory effect of the active compounds within these extracts is significant and efficacious in both cell free and cell-based systems in vitro.

As noted above, dual inhibitors of both the COX and 5-LO enzymes have distinct advantages over COX inhibitors alone. These advantages include, but are not limited to, a broader effect on inhibition of arachidonic acid metabolites, a lack of gastrointestinal toxicity and a potential decrease in vasocon-

striction. Therefore, the *Acacia* organic extracts were tested for their effects on 5-LO inhibition, as described in Example 8. Briefly, recombinant, human 5-LO was incubated with the *Acacia* extract for 15 minutes prior to the addition of enzyme substrate (umbelliferyl arachidonate). The results are set forth in FIG. **5**. The *Acacia* extracts inhibited 5-LO activity in this cell free in vitro system and demonstrated an IC_{50} of 70 μg/mL. Although the IC_{50} of the *Acacia* extracts for 5-LO is higher than that of the same extract for COX-2, it potentially adds a significant benefit for treating inflammation induced by arachidonic acid metabolism.

Two separate in vivo models were employed to determine whether the in vitro efficacy observed from the *Acacia* extracts translated to an ability to inhibit in vivo inflammatory responses. The two models are described in Example 9. The first of these systems is designed to measure inflammation resulting directly from the arachidonic acid metabolism pathway. In this example, mice were treated with extracts from *Acacia* prior to the topical application of AA to the ear to induce the inflammatory response. The effect of pretreating the animals was then measured by the inhibition of the ear swelling using a micrometer. The *Acacia* extracts demonstrated a good inhibitory response suggesting specific inhibition of AA induced inflammatory responses. The results are set forth in FIGS. **6**A and **6**B. Additionally, the extracts also showed significant inhibition when administered either orally or interperitoneally. Thus, the *Acacia* extracts were demonstrated to be efficacious in reducing inflammation by multiple routes of administration.

The organic extract from *Acacia* was the most efficacious against inflammation induced directly by the arachidonic acid. Therefore, the efficacy of a standardized *Acacia* extract was examined using a second model in which multiple mechanisms of inflammation are ultimately responsible for the final effect. This system is therefore more relevant to naturally occurring inflammatory responses. Using this model, a very potent activator of the complement system is injected into an air pouch created on the back of Balb/C mice. This results in a cascade of inflammatory events including, infiltration of inflammatory cells, activation of COX enzymes, resulting in the release of PGE_2, the enzyme myeloperoxidase (MPO), and production of a very specific profile of pro-inflammatory cytokines including TNF-α. These studies demonstrated that even though the *Acacia* did not inhibit the initial infiltration (chemotactic response) of inflammatory cells into the air pouch, it blocked the activation of those cells. This is evidenced by the lack of MPO excreted into the extracellular fluid of the pouch and the noted lack of production of TNF-α. The results are set forth in FIG. **7**. The data demonstrates that the *Acacia* extract is efficacious and helps control an inflammatory response in a model system where multiple inflammatory pathways are active. However, the standardized extract produced a toxic effect that was lethal to 60% of the animals receiving the highest concentration of *Acacia* extracts IP (200 mg/kg). This toxic effect was not observed in animals receiving the same dose by gavage.

The preparation of products for administration in pharmaceutical preparations may be performed by a variety of methods well known to those skilled in the art. The flavans may be formulated as an herb powder in the form of its natural existence; as solvent and/or supercritical fluid extracts in different concentrations; as enriched and purified compounds through recrystallization, column separation, solvent partition, precipitation and other means, as a pure and/or a mixture containing substantially purified flavans prepared by synthetic methods.

Various delivery systems are known in the art and can be used to administer the therapeutic compositions of the invention, e.g., aqueous solution, encapsulation in liposomes, microparticles, and microcapsules.

Therapeutic compositions of the invention may be administered parenterally by injection, although other effective administration forms, such as intraarticular injection, inhalant mists, orally active formulations, transdermal iontophoresis or suppositories are also envisioned. One preferred carrier is physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers may also be used. In one preferred embodiment, it is envisioned that the carrier and flavan(s) constitute a physiologically compatible, slow release formulation. The primary solvent in such a carrier may be either aqueous or non-aqueous in nature. In addition, the carrier may contain other pharmacologically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmacologically acceptable excipients for modifying or maintaining the stability, rate of dissolution, release or absorption of the ligand. Such excipients are those substances usually and customarily employed to formulate dosages for parental administration in either unit dose or multi-dose form.

Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder; or directly capsulated and/or tableted with other inert carriers for oral administration. Such formulations may be stored either in a ready to use form or requiring reconstitution immediately prior to administration. The manner of administering formulations containing the compositions for systemic delivery may be via oral, subcutaneous, intramuscular, intravenous, intranasal or vaginal or rectal suppository.

The amount of the composition which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder of condition, which can be determined by standard clinical techniques. In addition, in vitro or in vivo assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness or advancement of the disease or condition, and should be decided according to the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curved derived from in vitro or animal model test systems. For example, an effective amount of the composition of the invention is readily determined by administering graded doses of the composition of the invention and observing the desired effect.

The method of treatment according to this invention comprises administering internally or topically to a patient in need thereof a therapeutically effective amount of the individual and/or a mixture of multiple flavans from a single source or multiple sources. The purity of the individual and/or a mixture of multiple flavans includes, but is not limited to 0.01% to 100%, depending on the methodology used to obtain the compound(s). In a preferred embodiment doses of the flavans and pharmaceutical compositions containing the same are an efficacious, nontoxic quantity generally selected from the range of 0.01 to 200 mg/kg of body weight. Persons skilled in the art using routine clinical testing are able to determine optimum doses for the particular ailment being treated.

This invention includes an improved method for isolating and purifying flavans from *Acacia* plants, which is described in Example 10. In Example 10, flavans from *Acacia catechu* were extracted with different solvent systems. The results are

set forth in Table 6. The improved method of this invention comprises: extraction of the ground biomass of a plant containing flavans with an organic solvent or a combination of organic solvent(s) and/or water; neutralization and concentration of the neutralized extract; and purification of said extract by recrystallization and/or chromatography. It can be seen from Table 6, that 80% methanol in water is one of the preferred solvents for extraction of flavans from *Acacia* plants. As provided above, these flavans can be can be isolated from the *Acacia* genus of plants. The method of this invention can be extended to the isolation of these compounds from any plant source containing these compounds.

Additionally the flavans can be isolated from various parts of the plant including, but not limited to, the whole plant, stems, stem bark, twigs, tubers, flowers, fruit, roots, root barks, young shoots, seeds, rhizomes and aerial parts. In a preferred embodiment the flavans are isolated from the roots, reproductive organs or the whole plant.

The solvents that can be used for extraction of the ground biomass of the plant include, but are not limited to water, acidified water, water in combination with miscible hydroxylated organic solvent(s) including, but not limited to, methanol or ethanol and an mixture of alcohols with other organic solvent(s) such as THF, acetone, ethyl acetate etc. In one embodiment the extract is neutralized to a pH of 4.5-5.5 and then concentrated and dried to yield a powder. The flavans can then be purified by various methods including, but not limited to recrystallization, solvent partition, precipitation, sublimation, and/or chromatographic methods including, but not limited to, ion exchange chromatography, absorption chromatography, reverse phase chromatography, size exclusive chromatography, ultra-filtration or a combination of two.

The following examples are provided for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

Preparation of Organic and Aqueous Extracts from *Acacia catechu*

Plant material from *Acacia catechu* (L) Willd was ground to a particle size no larger than 2 mm. Dried ground plant material (60 g) was then transferred to an Erlenmeyer flask and methanol:dichloromethane (1:1) (600 mL) was added. The mixture was shaken for one hour, filtered and the biomass was extracted again with methanol:dichloromethane (1:1) (600 mL). The organic extracts were combined and evaporated under vacuum to provide the organic extract (see Table 1 below). After organic extraction, the biomass was air dried and extracted once with ultra pure water (600 mL). The aqueous solution was filtered and freeze-dried to provide the aqueous extract.

TABLE 1

Yield of Organic and Aqueous Extracts of *Acacia catechu*

Plant Source	Amount	Organic Extract	Aqueous Extract
Acacia catechu	60 g	27.2 g	10.8 g

Example 2

Inhibition of COX-2 and COX-1 Peroxidase Activity by Plant Extracts from *Acacia catechu*

The bioassay directed screening process for the identification of specific COX-2 inhibitors was designed to assay the peroxidase activity of the enzyme as described below.

Peroxidase Assay.

The assay to detect inhibitors of COX-2 was modified for a high throughput platform (Raz and Needleman (1990) J. Biol. Chem. 269:603-607). Briefly, recombinant ovine COX-2 (Cayman) in peroxidase buffer (100 mM, TBS, 5 mM EDTA, 1 μM Heme, 0.01 mg epinephrine, 0.094% phenol) was incubated with the extract (1:500 dilution) for 15 minutes. Quantablu (Pierce) substrate was added and allowed to develop for 45 minutes at 25° C. Luminescence was then read using a Wallac Victor 2 plate reader. The results are set forth in Table 2. The data in Table 2 is presented as the percent of peroxidase activity relative to the recombinant ovine COX-2 enzyme and substrate alone. The percent inhibition ranged from 30% (70% of control) for the aqueous extract to 75% for the organic extract. The data clearly demonstrates that the organic extract is the more efficacious in vitro.

TABLE 2

Inhibition of COX-2 Peroxidase activity by *Acacia catechu*

Plant Source	Inhibition of COX-2 by organic extract	Inhibition of COX-2 by aqueous extract
Acacia catechu	75%	30%

Comparison of the relative inhibition of the COX-1 and COX-2 isoforms requires the generation of IC_{50} values for each of these enzymes. The IC_{50} is defined as the concentration at which 50% inhibition of enzyme activity in relation to the control is achieved by a particular inhibitor. In the instant case, IC_{50} values were found to range from 3 to 6 μg/mL and 2.5 μg/mL for the COX-2 and COX-1 enzymes, respectively, as set forth in Table 3.

TABLE 3

IC_{50} Values for Human and Ovine COX-2 and COX-1

Plant Source	IC_{50} Human COX-2 (μg/mL)	IC_{50} Ovine COX-2 (μg/mL)	IC_{50} Ovine COX-1 (μg/mL)
Acacia catechu	3	6.25	2.5

Example 3

HTP Fractionation of Active Extracts

The organic extract (400 mg) from *Acacia catechu* was loaded onto a prepacked flash column. (2 cm ID×8.2 cm, 10 g silica gel). The column was eluted using an Hitachi high throughput purification (HTP) system with a gradient mobile phase of (A) 50:50 EtOAc:hexane and (B) methanol from 100% A to 100% B in 30 minutes at a flow rate of 5 mL/min. The separation was monitored using a broadband wavelength UV detector and the fractions were collected in a 96-deep-well plate at 1.9 mL/well using a Gilson fraction collector. The sample plate was dried under low vacuum and centrifu-

gation. DMSO (1.5 mL) was used to dissolve the samples in each cell and a portion (100 μL) was taken for the COX inhibition assay.

The aqueous extract (750 mg) from *Acacia catechu* was dissolved in water (5 mL), filtered through a 1 μm syringe filter and transferred to a 4 mL HPLC vial. The mixture was then injected by an autosampler onto a prepacked reverse phase column (C-18, 15 μm particle size, 2.5 cm ID×10 cm with precolumn insert). The column was eluted using an Hitachi high throughput purification (HTP) system with a gradient mobile phase of (A) water and (B) methanol from 100% A to 100% B in 20 minutes, followed by 100% methanol for 5 minutes at a flow rate of 10 mL/min. The separation was monitored using a broadband wavelength UV detector and the fractions were collected in a 96-deep-well plate at 1.9 mL/well using a Gilson fraction collector. The sample plate was freeze-dried. Ultra pure water (1.5 mL) was used to dissolve samples in each cell and a portion of 100 μL was taken for the COX inhibition assay. The active HTP fractions from well #C8 to F7 were analyzed with LC/MS/PDA at positive mode with super sonic ionization source. The results for active fraction D11 are set forth in FIG. **3**.

Example 4

Inhibition of COX Peroxidase Activity by HTP Fractions from *Acacia catechu*

Individual organic extracts were further characterized by examining each of the HTP fractions for the ability to inhibit the peroxidase activity of both COX-1 and COX-2 recombinant enzymes. The results are set forth in FIGS. **1** and **2**, which depicts the inhibition of COX-2 and COX-1 activity by HTP fractions from *Acacia* isolated as described in Example 1. The profile depicted in FIG. **1** shows a peak of inhibition that is very selective for COX-2 and multiple peaks that inhibit both enzymes with equal efficacy. However, both the COX-1 and COX-2 enzymes demonstrate multiple peaks of inhibition suggesting that there is more than one molecule contributing to the initial inhibition profiles.

Example 5

Isolation and Purification of the Active Compounds from the Organic Extract of *Acacia catechu*

The organic extract (5 g) from the roots of *Acacia catechu*, isolated as described in Example 1, was loaded onto prepacked flash column (120 g silica, 40 μm particle size 32-60 μm, 25 cm×4 cm) and eluted with a gradient mobile phase of (A) 50:50 EtOAc:hexane and (B) methanol from 100% A to 100% B in 60 minutes at a flow rate of 15 mL/min. The fractions were collected in test tubes at 10 mL/fraction. The solvent was evaporated under vacuum and the sample in each fraction was dissolved in DMSO (1 mL) and an aliquot of 20 μL was transferred to a 96 well shallow dish plate and tested for COX inhibitory activity. Based upon the COX assay results, active fractions #32 to #41 were combined and evaporated to yield 2.6 g of solid. Analysis by HPLC/PDA and LC/MS showed two major compounds with retention times of 15.8 and 16.1 minutes, respectively. The product was further purified on a C18 semi-preparatory column (25 cm×1 cm), loaded with 212.4 mg of product and eluted with a gradient mobile phase of (A) water and (B) acetonitrile (ACN), over a period of 60 minutes at a flow rate of 5 mL/minute. Eighty-eight fractions were collected and two active compounds were isolated. Compound 1 (11.5 mg) and Compound 2 (16.6 mg). Purity was determined by HPLC/PDA and LC/MS data by comparison with standards (catechin and epicatechin) and NMR data.

Compound 1.

^{13}C NMR: δ ppm (DMSO-d_6) 27.84 (C4), 66.27 (C3), 80.96 (C2), 93.78 (C9), 95.05 (C7), 99.00 (C5), 114.48 (C12), 115.01 (C15), 118.36 (C16), 130.55 (C11), 144.79 (C14), 155.31 (C6), 156.12 (C10), 156.41 (C8). ^{1}H NMR: δ ppm. (DMSO-d_6) 9.150 (1H, s, OH), 8.911 (1H, s, OH), 8.835 (1H, s, OH), 8.788 (1H, s, OH), 6.706 (1H, d, J=2 Hz, H2'), 6.670 (1H, d, J=8.0 Hz, H-6'), 6.578 (1H, dd, J=2, 8 Hz, H-5'), 5.873 (1H, d, J=2 Hz, H8), 5.670 (1H, d, J=2 Hz, H6), 4.839 (1H, d, J=4 Hz, OH), 4.461 (1H, d, J=7.3 Hz, H2), 3.798 (1H, m, H3), 2.625 (1H, m, H4b), 2.490 (1H, m, H4a). MS: $[M+1]^+$=291 m/e. This compound has been identified as catechin. The IC_{50} values of epicatechin against the COX-1 and COX-2 enzymes are 6 μg/mL and 40 μg/mL, respectively.

Compound 2.

^{13}C NMR: δ ppm. (DMSO-d_6) 28.17 (C4), 64.87 (C3), 78.02 (C2), 94.03 (C9), 95.02 (C7), 98.44 (C5), 114.70 (C12), 114.85 (C15), 117.90 (C16), 130.56 (C11), 144.39 (C14), 155.72 (C6), 156.19 (C10), 156.48 (C8). ^{1}H NMR: δ ppm. (DMSO-d_6) 9.083 (1H, s, OH), 8.873 (1H, s, OH), 8.777 (1H, s, OH), 8.694 (1H, s, OH), 6.876 (1H, d, J=2 Hz, H2'), 6.646 (2H, s, H-5', 6'), 5.876 (1H, d, J=2 Hz, H8), 5.700 (1H, d, J=2 Hz, H6), 4.718 (1H, s, OH), 4.640 (1H, d, J=4.5 Hz, H2), 3.987 (1H, d, J=4.5 Hz, H3), 2.663 (1H, dd, J=4.6, 6.3 Hz, H4b), 2.463 (1H, dd, J=4.6, 6.3 Hz, H4a). MS: $[M+1]^+$=2911n/e. This compound has been identified as epicatechin. The IC_{50} values of epicatechin against the COX-1 and COX-2 enzymes are 7 μg/mL and 20 μg/mL, respectively.

Example 6

HPLC Quantification of Active Extracts from *Acacia catechu*

The flavan content in the organic and aqueous extracts from *Acacia catechu* were quantified by High Pressure Liquid Chromatography (HPLC) using a PhotoDiode Array detector (HPLC/PDA) and a Luna C18 column (250 mm×4.6 mm). The flavans were eluted from the column using an acetonitrile gradient from 10% to 30% ACN over a period of 20 minutes, followed by 60% ACN for five minutes. The results are set forth in Table 4. The flavans were quantified based on retention time and PDA data using catechin and epicatechin as standards. The retention times for the two major flavans were 12.73 minutes and 15.76 minutes, respectively. The HPLC chromatograms are depicted in FIGS. **4**A and **4**B.

TABLE 4

Free-B-Ring Flavonoid Content in Active Plant Extracts

Active Extracts	Weight of Extract	% Extractible from BioMass	% Flavans in Extract
Acacia catechu (AE)*	10.8 g	18.0%	0.998%
Acacia catechu (OE)*	27.2 g	45.3%	30.37%

*AE: Aqueous Extract
*OE: Organic Extract

Example 7

In vitro Study of COX Inhibitory Activity of Organic Extracts from *Acacia catechu*

In vitro efficacy and COX-2 specificity of extracts from *Acacia catechu* were tested in cell based systems for their ability to inhibit the generation of arachidonic acid metabolites. Cell lines HOSC, which constitutively express COX-2 and THP-1, which express COX-1 were tested for their ability to generate prostaglandin E2 (PGE2) in the presence of arachidonic acid.

COX-2 Cell Based Assay.

HOSC (ATCC#8304-CRL) cells were cultured to 80-90% confluence. The cells were trypsinized, washed and resuspended in 10 mL at 1×10^6 cells/mL in tissue culture media (MEM). The cell suspension (200 μL) was plated out in 96 well tissue culture plates and incubated for 2 hours at 37° C. and 5% CO_2. The media was then replaced with new HOSC media containing 1 ng/mL IL-1b and incubated overnight. The media was removed again and replaced with 190 mL HOSC media. Test compounds were then added in 10 μL of HOSC media and were incubated for 15 minutes at 37° C. Arachidonic acid in HOSC media (20 mL, 100 μM) was added and the mixture was incubated for 10 minutes on a shaker at room temperature. Supernatant (20 μL) was transferred to new plates containing 190 μL/well of 100 μM indomethacin in ELISA buffer. The supernatants were analyzed as described below by ELISA.

COX-1 Cell Based Assay.

THP-1 cells were suspended to a volume of 30 mL (5×10^5 cells/mL). TPA was added to a final concentration of 10 nM TPA and cultured for 48 hours to differentiate cells to macrophage (adherent). The cells were resuspended in HBSS (25 mL) and added to 96 well plates in 200 mL volume at 5×10^5 cells/well. The test compounds in RPMI 1640 (10 μL) were then added and incubated for 15 minutes at 37° C. Arachidonic acid in RPMI (20 μL) was then added and the mixture was incubated for 10 minutes on a shaker at room temperature. Supernatant (20 μL) was added to Elisa buffer (190 μL) containing indomethacin (100 μM). The supernatants were then analyzed by ELISA, as described below.

COX-2 Whole Blood assay.

Peripheral blood from normal, healthy donors was collected by venipuncture. Whole blood (500 μL) was incubated with test compounds and extracts for 15 minutes at 37° C. Lipopolysaccharide (from *E. coli* serotype 0111:B4) was added to a final concentration of 100 μg/mL and cultured overnight at 37° C. The blood was centrifuged (12,000×g) and the plasma was collected. Plasma (100 μL) was added to methanol (400 μL) to precipitate proteins. Supernatants were measured for PGE2 production by ELISA. This procedure is a modification of the methods described by Brideau et al. (1996) Inflamm. Res. 45:68-74.

COX-1 Whole Blood Assay.

Fresh blood was collected in tubes not containing anticoagulants and immediately aliquoted into 500 μL aliquots in siliconized microcentrifuge tubes. Test samples were added, vortexed and allowed to clot for 1 hour at 37° C. The samples were then centrifuged (12,000×g) and the plasma was collected. The plasma (100 μL) was added to methanol (400 μL) to precipitate proteins. Supernatants were measured for TXB2 production by ELISA. This procedure is a modification of the methods described by Brideau et al. (1996) Inflamm. Res. 45:68-74.

ELISA Assays.

Immunolon-4 ELISA plates were coated with capture antibody 0.5-4 μg/mL in carbonate buffer (pH 9.2) overnight at 4° C. The plates were washed and incubated for 2 hours with blocking buffer (PBS+1% BSA) at room temperature. The plates were washed again and test sample (100 μL) was added and incubated for 1 hour at room temperature while shaking. Peroxidase conjugated secondary antibody was added in a 50 μL volume containing 0.5-4 mg/mL and incubated for 1 hour at room temperature while shaking. The plates were then washed three times and TMB substrate (100 μL) was added. The plates were allowed to develop for 30 minutes, after which the reaction was stopped by the addition of 1 M phosphoric acid (100 μL). The plates were then read at 450 nm using a Wallac Victor 2 plate reader.

Cytotoxicity.

Cellular cytotoxicity was assessed using a colorimetric kit (Oxford biochemical research) that measures the release of lactate dehydrogenase in damaged cells. Assays were completed according to manufacturers' directions. Both purified flavans and standardized extract from *Acacia catechu* were tested. No cytotoxicity was observed for any of the tested compounds.

The results of the assays are set forth in Table 5. The data is presented as IC_{50} values for direct comparison. With reference to Table 5, IC_{50} values are generally lower for COX-1 than COX-2. Additionally, whole blood was also measured for the differential inhibition of PGE2 generation (a measure of COX-2 in this system) or thromboxane B2 (TXB2) (a measure of COX 1 activation). Referring to Table 5, these studies clearly demonstrate specificity for COX-2 inhibition within the assays based on whole blood cells. However, studies using the THP-1 and HOSC based model system actually showed greater selectivity for COX-1. Possible reasons for this discrepancy are the fundamental differences between immortalized cell lines that constitutively express each of the enzymes and primary cells derived from whole blood that that are induced to express COX enzymes. Primary cells are the more relevant model to study inflammation in vivo. Additionally, the compounds used to identify COX-1 vs. COX-2 activity vary in each of these systems and consequently are not directly comparable.

TABLE 5

Inhibition of COX Activity in Whole Cell Systems

	Cell Line Based Assay		Whole Blood Assay	
Plant Source	IC_{50} COX-2	IC_{50} COX-1	IC_{50} COX-2	IC_{50} COX-1
Acacia catechu	78 μg/mL	22 μg/mL	40 μg/mL	>50 μg/mL

Example 8

Inhibition of 5 Lipoxygenase by the Organic Extracts from *Acacia catechu*

5-Lipoxygenase Assay.

Human recombinant 5-lipoxygenase enzyme (Cayman) was diluted (1:50) in assay buffer (50 mM Tris-HCl, pH 7.5, 2 mM $CaCl_2$, 1 mM ATP). Equal volumes of sample (1:500 dilution) and diluted enzyme were incubated together while shaking for 15 minutes. Substrate (umbelliferyl arachidonate, 1:5000 in assay buffer) was added and the mixture was incubated for 30 minutes at room temperature while shaking. The

resulting luminescence was read using a Wallac Victor 2 plate reader. The results are set forth in FIG. **5**.

Example 9

In Vivo Study of COX Inhibitory Activity of Organic Extracts from *Acacia catechu*

In vivo inhibition of inflammation was measured using two model systems. The first system (ear swelling model) measures inflammation induced directly by arachidonic acid. This is an excellent measure of COX-2 inhibition, but does not measure any of the cellular events which occur upstream of arachidonic acid liberation from cell membrane phospholipids by phospholipase A2 (PLA2). Therefore, to determine how inhibitors function in a more biologically relevant response the air pouch model was employed. This model utilizes a strong activator of complement to induce an inflammatory response that is characterized by a strong cellular infiltrate and inflammatory mediator production including cytokines as well as arachidonic acid metabolites.

Ear Swelling Model.

The ear swelling model is a direct measure of the inhibition of arachidonic acid metabolism. Briefly, arachidonic acid in acetone is applied topically to the ears of mice. The metabolism of arachidonic acid results in the production of proinflammatory mediators produced by the action of enzymes such as COX-2. Inhibition of the swelling is a direct measure of the inhibition of the enzymes involved in this pathway. The results are set forth in FIG. **6**, which shows the effects of the extracts delivered either orally by gavage or interperitoneally (IP) at two time points (24 hours and 1 hour). Extracts from *Acacia* inhibited swelling when delivered by both IP and gavage (FIG. **6**A and FIG. **6**B).

Air Pouch Model.

Because *Acacia* organic extracts were efficacious in the ear swelling model a standardized *Acacia* extract was also examined using the air pouch model of inflammation. Briefly, an air pouch was created on the back of mice by injecting 3 mL of sterile air. The air pouch was maintained by additional injections of 1 mL of sterile air every other day for a period of one week. Animals were dosed using the same methods and concentrations described for the ear-swelling model and injected with Zymosan (into the air pouch) to initiate the inflammatory response. After four hours, the fluid within the pouch was collected and measured for the infiltration of inflammatory cells, myeloperoxidase (MPO) activity (a measure of cellular activation, degranulation). The results are set forth in FIG. **7**.

FIG. **7**A shows the total number of cells collected from the air pouch fluid. While there was a strong response that was inhibited by controls (indomethacin), the standardized *Acacia* extract did not inhibit the infiltration of the inflammatory cells (chemotaxsis). Even though the chemotactic response was not diminished, the fluid was examined to determine whether the infiltrating cells have become activated by measuring MPO activity. FIGS. **7**B and **7**C demonstrate that MPO activity is significantly reduced when the extract is administered IP, but not by gavage. These data suggest that although the extracts do not inhibit the chemotactic response induced by complement activation, they are still effective at reducing inflammation through the prevention of release and production of pro-inflammatory mediators.

Arachidonic Acid Induced Ear Swelling.

The ability of *Acacia* extracts to directly inhibit inflammation in vivo was measured as previously described. (Greenspan et al. (1999) J. Med. Chem. 42:164-172; Young et al. (1984) J. Invest. Dermat. 82:367-371). Briefly, groups of 5 Balb/C mice were given dosages of test compounds either interperitoneally (I.P.) or orally by gavage, 24 hours and 1 hour prior to the application of arachidonic acid (AA). AA in acetone (2 mg/15 μL) was applied to the left ear, and acetone (15 μL) as a negative control was applied to the right ear. After 1 hour the animals were sacrificed by CO_2 inhalation and the thickness of the ears was measured using an engineer's micrometer. Controls included animals given AA, but not treated with anti-inflammatory agents and animals treated with AA and indomethacin (I.P.) at 5 mg/kg.

Air Pouch Model of Inflammation.

Air pouch models were adapted from the methods of Rioja et al. (2000) Eur. J. Pharm. 397:207-217. Air pouches were established in groups of 5 Balb/C mice by injection of sterile air (3 mL) and maintained by additional injections of 1 mL every 2 days for a period of six days. Animals were given dosages of test compounds either I.P. or orally by gavage, 24 hours and 1 hour prior to the injection of 1% Zymosan (1 mL) into the pouch. After 4 hours, the animals were sacrificed by CO_2 inhalation and the air pouches were lavaged with sterile saline (3 mL). The lavage fluid was centrifuged and the total number of infiltrating cells determined. Supernatants were also retained and analyzed for myleoperoxidase (MPO) activity and the presence of TNF-α by ELISA as measures of activation.

Example 10

Development a Standardized Extract from *Acacia catechu*

Acacia catechu (500 mg of ground bark) was extracted with the following solvent systems. (1) 100% water, (2) 80:20 water:methanol, (3) 60:40 water:methanol, (4) 40:60 water:methanol, (5) 20:80 water:methanol, (6) 100% methanol, (7) 80:20 methanol:THF, (8) 60:40 methanol:THF. The extract was concentrated and dried under low vacuum. Identification of the chemical components was carried out by High Pressure Liquid Chromatography using a PhotoDiode Array detector (HPLC/PDA) and a 250 mm×4.6 mm C18 column. The chemical components were quantified based on retention time and PDA data using catechin and epicatechin as standards. The results are set forth in Table 6 and FIG. **8**. As shown in Table 6 and FIG. **8**, the flavan extract generated from solvent extraction with 80% methanol/water provided the best concentration of flavan components.

TABLE 6

Solvents for Generating Standardized Flavan Extracts from *Acacia catechu*

Extraction Solvent	Weight of Extract	% Extractible from BioMass	Total amount of Catechins	% Catechins in Extract
100% water	292.8 mg	58.56%	0.13 mg	12.02%
water:methanol (80:20)	282.9 mg	56.58%	0.13 mg	11.19%
water:methanol (60:40)	287.6 mg	57.52%	0.15 mg	13.54%
water:methanol (40:60)	264.8 mg	52.96%	0.19 mg	13.70%
water:methanol (20:80)	222.8 mg	44.56%	0.15 mg	14.83%
100% methanol	215.0 mg	43.00%	0.15 mg	12.73%

TABLE 6-continued

Solvents for Generating Standardized Flavan Extracts from *Acacia catechu*

Extraction Solvent	Weight of Extract	% Extractible from BioMass	Total amount of Catechins	% Catechins in Extract
methanol:THF (80:20)	264.4 mg	52.88%	0.11 mg	8.81%
methanol:THF (60:40)	259.9 mg	51.98%	0.15 mg	9.05%

The invention claimed is:

1. A method for reducing joint discomfort, comprising administering to a human or non-human animal having joint discomfort a therapeutically effective amount of a composition comprising an *Acacia* extract enriched for flavans containing catechin and/or epicatechin, wherein the method alleviates cyclooxygenase and 5-lipoxygenase mediated inflammation in the human or non-human animal having joint discomfort.

2. The method according to claim **1**, wherein the *Acacia* is selected from the group consisting of *Acacia catechu, Acacia concinna, Acacia famesiana, Acacia senegal, Acacia speciosa, Acacia arabica, Acacia caesia, Acacia pennata, Acacia sinuata, Acacia meamsii, Acacia picnantha, Acacia dealbata, Acacia auriculiformis, Acacia holoserecia*, and *Acacia mangium*.

3. The method according to claim **1**, wherein the extract is from an *Acacia* plant part selected from the group consisting of stems, stem barks, trunks, trunk barks, twigs, tubers, roots, root barks, young shoots, seeds, rhizomes, flowers, and leaves.

4. The method according to claim **1**, wherein the cyclooxygenase and 5-lipoxygenase mediated inflammation is osteoarthritis, rheumatoid arthritis, or systemic lupus erythematosis.

5. The method according to claim **1**, wherein the *Acacia* extract is comprised of 0.01% to 100% of flavans.

6. The method according to claim **1**, wherein the *Acacia* extract is administered at a dose of 0.01 to 200 mg/kg of body weight of the human or non-human animal.

7. The method according to claim **1**, wherein the route of the administration is selected from the group consisting of oral, topical, suppository, intravenous, intradermic, intragaster, intramusclar, intraperitoneal, and intravenous.

8. The method according to claim **1**, wherein the extract is comprised of at least 14.83% catechin and epicatechin.

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